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PRIORITY SUBSTANCES LIST ASSESSMENT REPORT

1,3-Butadiene

(Revised August 2000)

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PRIORITY SUBSTANCES LIST ASSESSMENT REPORT

1,3-Butadiene

Environment Canada
Health Canada

May 2000
(Revised August 2000)

TABLE OF CONTENTS

SYNOPSIS 1

1.0 INTRODUCTION 3

2.0 SUMMARY OF INFORMATION CRITICAL TO ASSESSMENT OF "TOXIC" UNDER CEPA 1999 7

2.1 **Identity and physical/chemical properties** 7

2.2 **Entry characterization** 7
2.2.1 Production and uses 7
2.2.2 Sources and releases 8
2.2.2.1 Natural sources 8
2.2.2.2 Anthropogenic sources 8

2.3 **Exposure characterization** 9
2.3.1 Environmental fate 9
2.3.1.1 Air 9
2.3.1.2 Water 10
2.3.1.3 Sediment 10
2.3.1.4 Soil 10
2.3.1.5 Biota 10
2.3.1.6 Environmental distribution 11
2.3.2 Environmental concentrations 11
2.3.2.1 Ambient air 11
2.3.2.2 Indoor air 12
2.3.2.3 Surface water 13
2.3.2.4 Groundwater 13
2.3.2.5 Drinking water 13
2.3.2.6 Soil and sediment 14
2.3.2.7 Food 14
2.3.2.8 Consumer products 14

2.4 **Effects characterization** 14
2.4.1 Ecotoxicology 14
2.4.2 Toxicokinetics and metabolism 16
2.4.3 Experimental mammals and in vitro 20
2.4.3.1 Acute toxicity 20
2.4.3.2 Short-term and subchronic toxicity 20
2.4.3.3 Chronic toxicity and carcinogenicity 20
2.4.3.4 Genotoxicity 28
2.4.3.5 Reproductive and developmental toxicity 34
2.4.3.6 Immunotoxicity 35

2.4.4 *Humans* 37
2.4.4.1 Clinical studies 37
2.4.4.2 Carcinogenicity 37
2.4.4.3 Non-neoplastic effects 40
2.4.4.4 Genotoxicity 41
2.4.5 *Abiotic atmospheric effects* 42

3.0 ASSESSMENT OF "TOXIC" UNDER CEPA 1999 45

3.1 CEPA 1999 64(a): Environment 45
3.1.1 *Assessment and measurement endpoints* 45
3.1.1.1 Aquatic 45
3.1.1.2 Terrestrial 46
3.1.2 *Environmental risk characterization* 46
3.1.2.1 Aquatic effects 46
3.1.2.2 Terrestrial effects 47
3.1.2.2.1 Plants 47
3.1.2.2.2 Soil invertebrates 47
3.1.2.2.3 Mammalian wildlife 48
3.1.2.3 Discussion of uncertainty 50

3.2 CEPA 1999 64(b): Environment upon which life depends 50

3.3 CEPA 1999 64(c): Human health 50
3.3.1 *Estimated population exposure* 50
3.3.2 *Hazard characterization* 52
3.3.2.1 Weight of evidence for carcinogenicity and genotoxicity 52
3.3.2.2 Weight of evidence for non-neoplastic effects 55
3.3.3 *Exposure–response analyses* 56
3.3.3.1 Carcinogenicity 57
3.3.3.1.1 Estimated potency based on epidemiological data 57
3.3.3.1.2 Estimated potency based on data from studies in experimental animals 65
3.3.3.2 Non-neoplastic effects 70
3.3.4 *Human health risk characterization* 74
3.3.5 *Uncertainties and degree of confidence in human health risk characterization* 78

3.4 Conclusions 81

3.5 Considerations for follow-up (further action) 82

4.0 REFERENCES 83

APPENDIX A SEARCH STRATEGIES EMPLOYED FOR IDENTIFICATION OF RELEVANT DATA 103

List of Tables

Table 1 Chemical and physical properties of butadiene 7

Table 2 Acute and chronic environmental toxicity values for butadiene 15

Table 3 Incidences of neoplastic lesions in critical carcinogenicity bioassays for butadiene in experimental mammals 21

Table 4 Overview of genotoxicity of butadiene and its metabolites in rodents 29

Table 5 Summary of measures of risk for cancers of the lymphohematopoietic system in populations occupationally exposed to butadiene 36

Table 6 Summary of risk quotients for environmental assessment of butadiene 48

Table 7 Stratification variables for exposure–response modelling of epidemiological data from Delzell *et al.* (1995) 59

Table 8 Parameter estimates and model deviances for each of four models fitted to mean cumulative exposure per person-year for Delzell *et al.* (1995) study and comparison to parameter estimates from Delzell *et al.* analyses 62

Table 9 Carcinogenic potency estimates (TC_{01}s) for models fitted to mean cumulative exposure per person-year based on Delzell *et al.* (1995) study and comparison to estimates from Delzell *et al.* analyses 63

Table 10 Parameter estimates and model deviances for each of four lagged-exposure models fitted to median cumulative exposure per person-year 64

Table 11 Model validation p-values for Delzell *et al.* (1995) study 65

Table 12 Carcinogenic potency estimates (TC_{05}s) of butadiene based on results of bioassays in experimental animals 67

Table 13 Incidence and severity of ovarian atrophy observed in 2-year bioassay in mice 73

Table 14 Benchmark concentrations for ovarian atrophy 74

List of Figures

Figure 1 Proposed metabolism of butadiene in mammals 18

Figure 2 Observed rate ratios and fitted curves for leukemia in Delzell *et al.* (1995) study 61

Figure 3 Exposure–response analysis for butadiene-induced tumours in mice 68

Figure 4 Exposure–response analysis for butadiene-induced tumours in rats 71

Figure 5 Exposure–response analysis for ovarian atrophy in mice 73

Figure 6 Exposure–response analysis for ovarian atrophy in mice, excluding two highest dose groups 73

Figure 7 Exposure–response analysis for moderate/marked ovarian atrophy 74

Figure 8 Exposure–response analysis for moderate/marked ovarian atrophy, excluding high-dose group 74

List of Acronyms and Abbreviations

BMC_{05}	the concentration associated with a 5% increase in the benchmark endpoint
$BMCL_{05}$	the lower 95% confidence limit for the BMC_{05}
CAS	Chemical Abstracts Service
CEPA	*Canadian Environmental Protection Act*
CEPA 1999	*Canadian Environmental Protection Act, 1999*
CFC	chlorofluorocarbon
CI	confidence interval
CTV	Critical Toxicity Value
DEB	1,2,3,4-diepoxybutane
DNA	deoxyribonucleic acid
EB	1,2-epoxy-3-butene
EBdiol	1,2-dihydroxy-3,4-epoxybutane
EC_{50}	median effective concentration
EEV	Estimated Exposure Value
EH	epoxide hydrolase
ENEV	Estimated No-Effects Value
EPI	Exposure Potency Index
ETS	environmental tobacco smoke
GSH	glutathione
GT	glutathione transferase
GWP	Global Warming Potential
K_{ow}	octanol/water partition coefficient
LC_{50}	median lethal concentration
LCL	lower confidence limit
LOEC	Lowest-Observed-Effect Concentration
LOEL	Lowest-Observed-Effect Level
MATC	Maximum Acceptable Toxicant Concentration
MIR	Maximum incremental reactivity
NAPS	National Air Pollution Surveillance
NOEC	No-Observed-Effect Concentration
NO_X	nitrogen oxides
NPRI	National Pollutant Release Inventory
NTP	National Toxicology Program
O	observed cases
ODP	Ozone Depletion Potential
OR	odds ratio
PBPK	physiologically based pharmacokinetic
POCP	photochemical ozone creation potential
PSL	Priority Substances List

QSAR	quantitative structure–activity relationship
RR	relative risk
RTECS	Registry of Toxic Effects of Chemical Substances
SE	standard error
SMR	standardized mortality ratio
TC_{01}	tumorigenic concentration associated with a 1% increase in the incidence of or mortality due to cancer
TC_{05}	tumorigenic concentration associated with a 5% increase in the incidence of or mortality due to cancer
VOC	volatile organic compound

SYNOPSIS

1,3-Butadiene (hereafter referred to as butadiene) is a product of incomplete combustion resulting from natural processes and human activity. It is also an industrial chemical used primarily in the production of polymers, including polybutadiene, styrene-butadiene rubbers and latexes, and nitrile-butadiene rubbers. Butadiene enters the Canadian environment from exhaust emissions from gasoline- and diesel-powered vehicles, from non-transportation fuel combustion, from biomass combustion and from industrial on-site uses. The total amount of butadiene entering the Canadian environment was estimated to range from 12 917 to 41 622 tonnes in 1994, mostly into air.

While butadiene is not persistent, it is ubiquitous in the urban environment because of its widespread combustion sources. Highest atmospheric concentrations have been measured in air in cities and close to an industrial source. Given its sources of entry into the environment, its environmental fate and concentrations measured in Canada, the environmental assessment focussed on assessing the potential risks to aquatic life, terrestrial plants, terrestrial wildlife and soil invertebrates. The potential risks were assessed assuming worst-case, hyperconservative conditions. Analyses indicate that environmental biota are unlikely to be at risk even under such conditions.

Because of its non-halogenated nature and moderate environmental concentrations, butadiene is not associated with stratospheric ozone depletion or with climate change. Butadiene is a reactive chemical with a high photochemical ozone creation potential and moderate concentrations in air, and therefore is a contributor to the formation of ground-level ozone and resulting smog formation.

The general population in Canada is exposed to butadiene primarily through ambient and indoor air. Inhaled butadiene is carcinogenic in both mice and rats, inducing tumours at multiple sites at all concentrations tested in all identified studies. In addition, butadiene is genotoxic in both somatic and germ cells of rodents. The greater sensitivity in mice than in rats to induction of these effects by butadiene is likely related to species differences in metabolism to active epoxide metabolites. An association between exposure to butadiene in the occupational environment and leukemia fulfils several of the traditional criteria for causality; there is also some limited evidence that butadiene is genotoxic in exposed workers. Therefore, in view of the weight of evidence of available epidemiological and toxicological data, butadiene is considered highly likely to be carcinogenic in humans; it is also considered likely to be genotoxic in humans. Butadiene also induced adverse effects in the reproductive organs of female mice at relatively low concentrations.

Based on the information available, it is concluded that butadiene is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. However, butadiene is concluded to be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends and a danger in Canada to life or health. Therefore, butadiene is considered to be "toxic" as defined in Section 64 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999).

Butadiene contributes to the photochemical formation of ground-level ozone. It is recommended that key sources of butadiene be addressed, therefore, as part of management plans for volatile organic chemicals that contribute to the formation of ground-level ozone.

Based on comparison of estimates of exposure for the general population with the tumorigenic potency, the priority to investigate options to reduce exposure to butadiene in ambient air in the vicinity of the identified point sources is considered to be high, while that from more dispersive non-point sources (identified herein primarily as transportation) is considered to be moderate to high. Investigation of concentrations and potential sources of butadiene in indoor air may also be warranted.

1.0 INTRODUCTION

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) requires the federal Ministers of the Environment and of Health to prepare and publish a Priority Substances List (PSL) that identifies substances, including chemicals, groups of chemicals, effluents and wastes, that may be harmful to the environment or constitute a danger to human health. The Act also requires both Ministers to assess these substances and determine whether they are "toxic" or are capable of becoming "toxic" as defined in Section 64 of the Act, which states:

> ...a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that
> (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
> (b) constitute or may constitute a danger to the environment on which life depends; or
> (c) constitute or may constitute a danger in Canada to human life or health.

Substances that are assessed as "toxic" as defined in Section 64 may be placed on Schedule I of the Act and considered for possible risk management measures, such as regulations, guidelines, pollution prevention plans or codes of practice, to control any aspect of their life cycle, from the research and development stage through manufacture, use, storage, transport and ultimate disposal.

Based on initial screening of readily accessible information, the rationale for assessing 1,3-butadiene (hereafter referred to as butadiene) provided by the Ministers' Expert Advisory Panel on the Second Priority Substances List (Ministers' Expert Advisory Panel, 1995) was as follows:

> 1,3-Butadiene is present at low levels in indoor and outdoor air throughout the country. Sources include motor vehicle emissions and the manufacture of plastics and synthetic rubbers. The substance is carcinogenic and genotoxic in animals. It may be carcinogenic in humans. It is important to assess the potential risk to human health and the environment.

Descriptions of the approaches to assessment of the effects of Priority Substances on the environment and human health are available in published companion documents. The document entitled "Environmental Assessments of Priority Substances under the *Canadian Environmental Protection Act*. Guidance Manual Version 1.0 — March 1997" (Environment Canada, 1997a) has been published to provide guidance for conducting environmental assessments of Priority Substances in Canada. This document may be purchased from:

> Environmental Protection Publications
> Environmental Technology Advancement Directorate
> Environment Canada
> Ottawa, Ontario
> K1A 0H3

It is also available on the Internet at www.ec.gc.ca/cceb1/eng/psap.htm under the heading "Technical Guidance Manual." It should be noted that the approach outlined therein has evolved to incorporate recent developments in risk assessment methodology, which will be addressed in future releases of the guidance manual for environmental assessments of Priority Substances.

The approach to assessment of effects on human health is outlined in the following publication of the Environmental Health Directorate of Health Canada: "*Canadian Environmental Protection Act* — Human Health Risk Assessment for Priority Substances" (Health Canada, 1994), copies of which are available from:

> Environmental Health Centre
> Room 104
> Health Canada
> Tunney's Pasture
> Ottawa, Ontario
> K1A 0L2

or on the Environmental Health Directorate publications web site — www.hc-sc.gc.ca/ehp/ehd/catalogue/bch.htm. The approach is also described in an article published in the *Journal of Environmental Science and Health — Environmental Carcinogenesis and Ecotoxicology Reviews* (Meek *et al.*, 1994). It should be noted that the approach outlined therein has evolved to incorporate recent developments in risk assessment methodology, which are described on the Environmental Substances Division web site — www.hcsc.gc.ca/ehp/ehd/bch/env_contaminants/psap/psap.htm — and which will be addressed in future releases of the approach paper for the assessment of effects on human health.

The search strategies employed in the identification of data relevant to the assessment of entry, environmental fate and exposure and potential effects on the environment (prior to March 1998) and human health (prior to April 1998 for toxicity information) are presented in Appendix A. Although much of the research on butadiene has been conducted outside Canada, available data on sources, use patterns and fate of butadiene in the Canadian environment have been emphasized. Review articles were consulted where appropriate. However, all original studies that form the basis for determining whether butadiene is "toxic" under CEPA have been critically evaluated by staff of Environment Canada (entry and environmental exposure and effects) and Health Canada (human exposure and effects on human health).

Sections of the Assessment Report and the supporting documentation (Environment Canada, 1998) related to the environmental assessment of butadiene were prepared or reviewed by the members of the Environmental Resource Group established by Environment Canada to support the environmental assessment:

A. Bobra, AMBEC Environmental Consultants (coordinator for the environmental assessment)
Y. Bovet, Environment Canada
N. Bunce, University of Guelph
R. Chénier, Environment Canada (lead for the environmental assessment)
T. Dann, Environment Canada
F. Edgecomb, Canadian Plastics Industry Association
P. Georges, Environment Canada
R. Keefe, Imperial Oil Ltd.
F. Onuska, Environment Canada
F. Ratpan, Nova Chemicals
G. Rideout, Environment Canada
A. Stelzig, Environment Canada
M. Tushingham, Environment Canada
C.J. West, Bayer Rubber Inc.

The sections of the Assessment Report and supporting documentation relevant to the environmental assessment were also reviewed by:

S. Abernethy, Ontario Ministry of the Environment
L. Brownlee, Environment Canada
P. Makar, Environment Canada
L. McCarty, L.S. McCarty Scientific Research & Consulting (who also prepared a first draft of the environmental sections of the Assessment Report)
S. Robertson, U.K. Environment Agency
J. Schaum, U.S. Environmental Protection Agency
A. Sergeant, U.S. Environmental Protection Agency
G. Whitten, Systems Application International

Sections of this Assessment Report and supporting documentation related to health were prepared, based, in part, on background information prepared in 1994 by BIBRA International, by the following staff of Health Canada:

R. Beauchamp
K. Hughes
M.E. Meek
D. Moir
M. Walker

Sections of the Assessment Report and supporting documentation on genotoxicity and reproductive and developmental toxicity were reviewed by D. Blakey and W. Foster, respectively, of the Environmental and Occupational Toxicology Division of Health Canada. A review of the exposure assessment included in the critical epidemiological studies was prepared under contract by M. Gerin and J. Siemiatycki of the Institut Armand-Frappier, University of Quebec.

In the first stage of external review, sections of the supporting documentation pertaining to human health were considered by the following individuals, primarily to address adequacy of coverage:

J. Aquavella, Monsanto Company
M. Bird, Exxon Biomedical Sciences, Inc.
J.A. Bond, Chemical Industry Institute of Toxicology
I. Brooke, U.K. Health and Safety Executive
G. Granville, Shell Canada Ltd.
R. Keefe, Imperial Oil Ltd.
A. Koppikar, U.S. Environmental Protection Agency
R.J. Lewis, Exxon Biomedical Sciences, Inc.
K. Peltonen, Finnish Institute of Occupational Health
F. Ratpan, Nova Chemicals

In the second stage of external review, accuracy of reporting, adequacy of coverage and defensibility of conclusions with respect to hazard characterization and exposure–response analyses were considered in written review by BIBRA International and the following individuals:

R.J. Albertini, University of Vermont
J.A. Bond, Chemical Industry Institute of Toxicology
I. Brooke, U.K. Health and Safety Executive
J. Bucher, U.S. National Toxicology Program
B. Davis, U.S. National Toxicology Program
E. Delzell, University of Alabama at Birmingham
B.J. Divine, Texaco
A.A. Elfarra, University of Wisconsin-Madison
E. Frome, Oak Ridge National Laboratory
B.D. Goldstein, Environmental and Occupational Health Sciences Institute
R.F. Henderson, Lovelace Respiratory Research Institute
R.D. Irons, University of Colorado Health Sciences Center
A. Koppikar, U.S. Environmental Protection Agency
J. Lubin, National Cancer Institute
J. Lynch, Exxon Biomedical Sciences, Inc. (retired)
R.L. Melnick, U.S. National Toxicology Program
K. Peltonen, Finnish Institute of Occupational Health
A.G. Renwick, University of Southampton
J. Siemiatycki, Institut Armand-Frappier
L.T. Stayner, U.S. National Institute for Occupational Safety and Health
J.A. Swenberg, University of North Carolina
R. Tice, Integrated Laboratory Systems, Inc.
J.B. Ward, Jr., University of Texas Medical Branch

In the third and final stage of external review, adequacy of incorporation of the comments received during the second stage was considered at a final meeting of a panel of the following members convened by Toxicology Excellence in Risk Assessment (TERA) in November 1998:

H. Clewell, K.S. Crump Division of ICF Kaiser
M.L. Dourson, TERA
L. Erdreich, Bailey Research Associates, Inc.

The health-related sections of the Assessment Report were reviewed and approved by the Health Protection Branch Risk Management meeting.

The entire Assessment Report was reviewed and approved by the Environment Canada/Health Canada CEPA Management Committee.

A draft of the Assessment Report was made available for a 60-day public comment period (October 2 to December 1, 1999) (Environment Canada and Health Canada, 1999). Following consideration of comments received, the Assessment Report was revised as appropriate. A summary of the comments and their responses is available on the Internet at:

www.ec.gc.ca/cceb1/eng/final/index_e.html

The text of the Assessment Report has been structured to address environmental effects initially (relevant to determination of "toxic" under Paragraphs 64(a) and (b)), followed by effects on human health (relevant to determination of "toxic" under Paragraph 64(c)).

Copies of this Assessment Report are available upon request from:

Inquiry Centre
Environment Canada
Main Floor, Place Vincent Massey
351 St. Joseph Blvd.
Hull, Quebec
K1A 0H3

or on the Internet at:

www.ec.gc.ca/cceb1/eng/final/index_e.html

Unpublished supporting documentation, which presents additional information, is available upon request from:

Commercial Chemicals Evaluation Branch
Environment Canada
14th Floor, Place Vincent Massey
351 St. Joseph Blvd.
Hull, Quebec
K1A 0H3

or

Environmental Health Centre
Room 104
Health Canada
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

2.0 Summary of Information Critical to Assessment of "Toxic" under CEPA 1999

2.1 Identity and physical/chemical properties

1,3-Butadiene is also known as butadiene, alpha-gamma-butadiene, buta-1,3-diene, bivinyl, divinyl, erythrene, vinylethylene, biethylene and pyrrolylene. Its Chemical Abstracts Service (CAS) registry number is 106-99-0, and its Registry of Toxic Effects of Chemical Substances (RTECS) number is EI9275000.

The chemical and physical characteristics of butadiene are shown in Table 1. Ranges of reported values have been discussed by Mackay *et al.* (1993) and are presented in the environmental Supporting Document (Environment Canada, 1998). At room temperature, butadiene is a colourless, flammable gas with a mild aromatic odour. It has a high vapour pressure, a moderately low water solubility and a low octanol/water partition coefficient (K_{ow}) (Mackay *et al.*, 1993).

2.2 Entry characterization

2.2.1 Production and uses

Butadiene is produced during the combustion of organic matter in both natural processes and human activities. In addition, it is produced commercially for use in the chemical polymer industry.

Table 1 Chemical and physical properties of butadiene

Property	Value	Reference
Molecular formula	C_4H_6	
Structural formula	CH_2=CH-CH=CH_2	
Molecular weight	54.09 g/mol	Mackay *et al.*, 1993
Physical state	colourless gas at 25°C with a mild aromatic odour	Mackay *et al.*, 1993
Boiling point	-4.44°C	Mackay *et al.*, 1993
Melting point	-108.9°C	Weast, 1984
Water solubility (experimental, at 25°C and 101.325 kPa)	735 mg/L	McAuliffe, 1966
Vapour pressure (calculated, at 25°C)	281 kPa	Mackay *et al.*, 1993
Absorption spectrum	insignificant above 230 nm	Jaber *et al.*, 1984
Henry's law constant (calculated, at 25°C and 101.325 kPa)	7460 Pa·m^3/mol	Mackay and Shiu, 1981
Log octanol/water partition coefficient (log K_{ow}) (experimental)	1.99	Leo *et al.*, 1975
Log organic carbon partition coefficient (log K_{oc}) (calculated)	1.86–2.36	Lyman *et al.*, 1982

In 1994, there was one Canadian commercial producer of butadiene (located in Sarnia, Ontario), with a production capacity of 120 kilotonnes and actual domestic production of 103.7 kilotonnes. Butadiene is purified by an extraction distillation process from a crude petroleum butadiene stream. Importation into Canada from the United States was 1.7 kilotonnes in 1994. The Canadian domestic use of butadiene in 1994 amounted to 105.4 kilotonnes (98.3 kilotonnes for total domestic demand and 7.1 for export sales) (Camford Information Services, 1995).

The largest end use of butadiene in Canada is the production of polybutadiene rubber (51.4 kilotonnes; 52.3% of total Canadian consumption for 1994) (Camford Information Services, 1995). Other derivatives produced include styrene-butadiene latexes (31.0 kilotonnes; 31.5% of total Canadian consumption for 1994), nitrile-butadiene rubbers (10.0 kilotonnes; 10.2% for 1994), acrylonitrile-butadiene-styrene terpolymer (3.4 kilotonnes; 3.5% for 1994) and specialty styrene-butadiene rubbers (2.5 kilotonnes; 2.5% of total Canadian consumption for 1994).

Results from a survey of industry carried out under the authority of Section 16 of CEPA indicated generally similar commercial data for 1996 (Environment Canada, 1997c).

Butadiene has a long history of use, notably related to production of polymers. Several industrial and commercial products are manufactured with it or may contain it as a component. Examples include tires, car sealants, plastic bottles and food wrap, epoxy resins, lubricating oils, hoses, drive belts, moulded rubber goods, adhesives, paint, latex foams for carpet backing or underpad, shoe soles, moulded toys/household goods, medical devices and chewing gum (CEH-SRI International, 1994; OECD, 1996).

2.2.2 *Sources and releases*

Estimates of emissions are highly variable, depending on the method of estimation and the quality of the data upon which they are based. Total Canadian emissions for 1994 were estimated to range between 12 917 and 41 622 tonnes (Environment Canada, 1998). Major uncertainties are associated with estimates for combustion sources, notably forest fires.

2.2.2.1 Natural sources

Butadiene is released from biomass combustion, especially forest fires. Total global emission of butadiene from biomass combustion was estimated to be 770 000 tonnes per year (Ward and Hao, 1992). Releases from forest fires in Canada were estimated to range between 3607 and 26 966 tonnes, which constituted 49.3% (range of estimates is 28–65%) of the total annual emissions of butadiene in Canada (CPPI, 1997). Forest fires are sporadic events, both in time and space. As indicated below, the atmospheric half-life of butadiene is short (hours). Thus, while forest fires are an important local source of butadiene soon after their occurrence, they likely contribute little to the concentrations consistently measured in urban or industrial areas.

2.2.2.2 Anthropogenic sources

All internal combustion engines may produce butadiene as a result of incomplete combustion. The amount generated and released depends primarily on the composition of fuel, the type of engine, the emission control used (i.e., presence and efficiency of catalytic converter), the operating temperature, and the age and state of repair of the vehicle (Environment Canada, 1996a). Cyclohexane, 1-hexene, 1-pentene and cyclohexene have been identified as primary fuel precursors for butadiene (Schuetzle *et al.*, 1994). As well, very low levels of butadiene itself may be present in gasoline and in liquefied petroleum gas (Environment Canada, 1998).

Butadiene can also enter the environment from any stage in the production, storage, use, transport or disposal of products with residual, free or unreacted butadiene. Data on Canadian industrial emissions have been collected for

industrial processes, plastic products industries, refined petroleum and coal products industries, and chemical and chemical products industries as part of the National Pollutant Release Inventory (NPRI) (Environment Canada, 1996b, 1997d). Emissions other than those reported to the NPRI may occur, including from combustion of other fuels (e.g., natural gas, oil and wood), prescribed forest burning, cigarettes, waste incineration, releases from polymer products, releases from the use and disposal of products containing butadiene, and spillage (Ligocki *et al.*, 1994; Environment Canada, 1996c; OECD, 1996).

The following amounts of butadiene were estimated to have been released into the Canadian environment in 1994 from transportation and related sources (Environment Canada, 1996b; CPPI, 1997): 3376–7401 tonnes from on-road gasoline- and diesel-powered motor vehicles (with about 45–89% of those releases from gasoline engines and 11–55% from diesel engines); 150–258 tonnes from aircraft; 84–1689 tonnes from off-road motor vehicles; 84 tonnes from lawnmowers; 40 tonnes from the marine sector; and 17 tonnes from the rail sector. Data on releases from on-road motor vehicles in 1994 were estimated by modelling (Mobile 5C model), using assumptions outlined in Environment Canada (1996b). It can be expected that the rates of release of butadiene from automotive sources have and will continue to change; most current and planned modifications to automotive emission control technology and gasoline quality would lead to decreases in the releases of butadiene and other VOCs.

In addition, data from NPRI for 1994 (Environment Canada, 1996b) listed a total of 270.4 tonnes released from the chemical and chemical products industries. Of this, 270.3 tonnes were released into air, 0.058 tonnes into water (St. Clair River, Ontario) and 0.002 tonnes onto land. There were releases of 17.5 tonnes into air from the plastic products industries. A total of 22.3 tonnes was released from the refined petroleum and coal products industries, of which 22.2 tonnes were released into air. Off-site transfer of wastes (material sent for final disposal or treatment prior to final disposal) from industrial sites in Canada in 1994 was estimated to include a total of 131.3 tonnes of butadiene, with 128.7 tonnes being sent to incineration, 2.1 tonnes to landfill and 0.5 tonnes to municipal sewage treatment plants (Environment Canada, 1996b). Based on 1995 NPRI data (Environment Canada, 1997d), the amount of butadiene estimated to have been released into the Canadian environment was 225.8 tonnes from industrial on-site uses, with 0.058 tonnes released into water, 0.002 tonnes into land and 225.4 tonnes into air. Releases into air included air fugitive releases (172.8 tonnes), air stack releases (36.3 tonnes), air storage releases (4.8 tonnes), air spill releases (1.1 tonnes) and other air releases (10.4 tonnes).

Based on data in NPRI, it was estimated that the total release of butadiene from fuel distribution in 1994 was 24 tonnes (Environment Canada, 1996b), although gasoline and diesel fuel contain little butadiene.

CPPI (1997) estimated that releases into the Canadian environment in 1994 were 1191 tonnes from prescribed forest burning, 3706 tonnes from wood space heating, 11 tonnes from natural gas/oil space heating and 1–9 tonnes from cigarettes.

2.3 Exposure characterization

2.3.1 Environmental fate

2.3.1.1 Air

Since butadiene is released primarily to air, its fate in that medium is of primary importance. Butadiene is not expected to persist in air, since it oxidizes rapidly with several oxidant species. Destruction of atmospheric butadiene by the gas-phase reaction with photochemically produced hydroxyl radicals is expected to be the dominant photo-initiated pathway. Products that can be formed include formaldehyde, acrolein and furan. Destruction by nitrate radicals is expected

to be a nighttime process in urban areas. Acrolein, trans-4-nitroxy-2-butenal and 1-nitroxy-3-buten-2-one have been identified as products of this reaction. Reaction with ozone is also rapid but less important than reaction with hydroxyl radicals. The products from the reaction of butadiene with ozone are acrolein, formaldehyde, acetylene, ethylene, formic acid, formic anhydride, carbon monoxide, carbon dioxide, hydrogen gas, hydroperoxyl radical, hydroxyl radical and 3,4-epoxy-1-butene (Atkinson *et al.*, 1990; Howard *et al.*, 1991; McKone *et al.*, 1993; U.S. EPA, 1993).

Estimated average atmospheric half-lives for photo-oxidation of butadiene range from 0.24 to 10 hours (Darnell *et al.*, 1976; Lyman *et al.*, 1982; Atkinson *et al.*, 1984; Howard *et al.*, 1991; Mackay *et al.*, 1993). However, half-lives for butadiene in air can vary considerably under different conditions. Estimations for atmospheric residence time in several U.S. cities ranged from 0.4 hours under clear skies at night in the summer to 2000 hours (83 days) under cloudy skies at night in the winter. Daytime residence times for different cities within a given season varied by factors of 2–3. Nighttime residence times varied by larger factors. The differences between summer and winter conditions were large at all sites, with winter residence times 10–30 times greater than summer residence times (U.S. EPA, 1993). Because of the long residence times under some conditions, especially in winter under cloudy conditions, there is a possibility of day-to-day carryover. Nonetheless, given the generally short daytime residence times, the net atmospheric lifetime of butadiene is short, and there is generally limited potential for long-range transport of this compound.

It is predicted from its physical/chemical properties that when butadiene is released into air, almost all of it will exist in the vapour phase in the atmosphere (Eisenreich *et al.*, 1981; Environment Canada, 1998). Wet and dry deposition are not expected to be important as transfer processes. Evaporation from rain may be rapid, and the compound is returned to the atmosphere relatively quickly unless it is leached into the soil.

2.3.1.2 Water

Volatilization, biodegradation and oxidation by singlet oxygen are the most prominent processes involved in determining the fate of butadiene in water. The estimated half-lives of butadiene by reaction in water range from 4.2 to 28 days (Howard *et al.*, 1991; Mackay *et al.*, 1993).

2.3.1.3 Sediment

The processes that are most prominent in determining the environmental fate of butadiene in sediment are biotic and abiotic degradation. The estimated half-lives of butadiene by reaction in sediment range from 41.7 to 125 days (Mackay *et al.*, 1993).

2.3.1.4 Soil

Based on its vapour pressure and its solubility, volatilization of butadiene from soil and other surfaces is expected to be significant. Butadiene's organic carbon/water partition coefficient indicates that it should not adsorb to soil particles to a great degree and would be considered moderately mobile (Kenaga, 1980; Swann *et al.*, 1983). However, the rapid rate of volatilization and the potential for degradation in soil suggest that it is unlikely that butadiene will leach into groundwater. The estimated half-life of butadiene by reaction, given by Howard *et al.* (1991) and Mackay *et al.* (1993), ranges from 7 to 41.7 days.

2.3.1.5 Biota

There are no measured bioconcentration factors. Butadiene is metabolized by the mixed-function oxidase system in higher organisms, which contributes to the expected lack of accumulation by many organisms. Estimated bioconcentration factors for butadiene in fish have been reported to range from 4.6 to 19 (Lyman *et al.*, 1982; OECD, 1996). Even though estimation methods likely overestimate the true bioconcentration potential

for a readily metabolized substance, they indicate that butadiene is not expected to bioconcentrate in aquatic organisms or to biomagnify in the aquatic food chain.

There are no reported measurements of plant-root bioconcentration in soils. However, McKone *et al.* (1993) estimated the uptake of butadiene by roots from soil solution to be 1.84 L/kg, which is the ratio of butadiene concentration in root (mg/kg, fresh mass) to concentration in soil solution (mg/L). The partition coefficient of butadiene concentration in roots (mg/kg, fresh mass) to concentration in soil solids (mg/kg) was estimated to range from 0.32 to 15 (dimensionless). The partition coefficient of butadiene concentration in whole plants (mg/kg, fresh mass) to concentration in soil solids (mg/kg) was estimated to range from 0.1 to 2.9 (dimensionless). The steady-state plant/air partition coefficient for foliar uptake of butadiene in plant leaves was estimated to be 0.63 m^3/kg. There are no reported bioaccumulation data for any terrestrial invertebrates.

2.3.1.6 Environmental distribution

Fugacity modelling was conducted to provide an overview of key reaction, intercompartment and advection (movement out of a system) pathways for butadiene and of overall distribution in the environment. A steady-state, non-equilibrium model (Level III fugacity modelling) was run using the methods developed by Mackay (1991) and Mackay and Paterson (1991). Assumptions, input parameters and results are presented in Environment Canada (1998).

Based on butadiene's physical/chemical properties, Level III fugacity modelling predicts that (Environment Canada, 1998):

- when butadiene is released into air, the distribution of mass is almost 100% in air, with very small amounts in soil and water;
- when butadiene is released into water, the distribution of mass is 98.1% in water, with small amounts in air;
- when butadiene is released into soil, the distribution of mass is 47.6% in soil, 51.5% in air and 0.9% in water.

Modelling predictions do not purport to reflect actual expected measurements in the environment but rather indicate the broad characteristics of the fate of the substance in the environment and its general distribution between media. Thus, when butadiene is discharged into air or water, most of it is expected to be found in the medium receiving the discharge directly. For example, if butadiene is discharged into air, almost all of it will exist in the atmosphere, where it will react rapidly and will also be transported away. If butadiene is discharged to water, it will react in water, and some will also evaporate into air. If butadiene is discharged to soil, most will be present in air or soil, where it will react (Mackay *et al.*, 1993; Environment Canada, 1998).

2.3.2 Environmental concentrations

2.3.2.1 Ambient air

Butadiene was detected (detection limit 0.05 $\mu g/m^3$) in 7314 (or 80%) of 9168 24-hour samples collected between 1989 and 1996 under the National Air Pollution Surveillance (NAPS) program from rural, suburban and urban locations (n = 47) in seven provinces (Dann, 1997). The mean concentration in all samples was 0.3 $\mu g/m^3$ (in the calculation of the mean, a value of one-half the detection limit was assumed for samples in which levels were below the detection limit), and the maximum concentration measured was 14.1 $\mu g/m^3$. Concentrations of butadiene in ambient air corresponding to the 50th and 95th percentiles of the NAPS data set were 0.21 and 1.0 $\mu g/m^3$, respectively. There was a seasonal variation in the mean concentration of butadiene in ambient air, with levels being lower during the late spring and early summer months. This seasonal variation in mean concentration was more pronounced for suburban NAPS sites than for urban sites. There is no evidence that concentrations of butadiene in ambient air in Canada have been increasing or decreasing in a systematic manner during the 1990s.

Concentrations of butadiene were determined in 1611 samples of outdoor air from 25 sites within 14 cities, towns or rural locations in Ontario between 1990 and 1994 (Steer, 1996). Butadiene was detected in 37% of the samples (detection limits 0.04–0.1 μg/m³). The frequency of detection was much higher at downtown sites than at rural sites. The mean concentration in all samples was 0.1 μg/m³ (in the calculation of the mean, a value of one-half the detection limit was assumed for samples in which levels were below the detection limit), and the maximum concentration measured was 1.7 μg/m³. Concentrations of butadiene in ambient air corresponding to the 50th and 95th percentiles of this data set were 0.05 and 0.3 μg/m³, respectively.

Butadiene has been detected at concentrations generally less than 2 μg/m³ in a low percentage of samples of outdoor air collected during several small studies conducted during the 1990s in Toronto (Bell *et al.*, 1991), Windsor (Bell *et al.*, 1993) and Hamilton (Hamilton-Wentworth, 1997) in Ontario and in Edmonton and Fort Saskatchewan, Alberta (Conor Pacific Environmental, 1998). The highest reported concentration of butadiene in outdoor air in Canada (i.e., 28 μg/m³ in a 30-minute sample) was measured in 1995 within 1 km of an industrial point source of discharge to the atmosphere in Sarnia, Ontario (MOEE, 1995). Butadiene was detected in 78% of samples collected at various distances downwind from the point source, but in only 38% of samples collected upwind (detection limit 0.11 μg/m³ for 30-minute air samples). Concentrations decreased with distance from the source. At distances between 1 and 3 km downwind of the source, the concentrations of butadiene in ambient air corresponding to the 50th and 95th percentiles of this data set were 0.62 and 6.4 μg/m³, respectively, while the levels corresponding to the 50th and 95th percentiles of samples at distances of 1 km and greater (upper end of the range not specified) were 0.48 and 2.6 μg/m³, respectively.

Butadiene has also been detected in air in enclosed structures. Concentrations of butadiene between 4 and 49 μg/m³ were measured during the winter months of 1994–95 in Canadian underground parking garages (Environment Canada, 1994) because of its presence in vehicle exhaust. Similarly, butadiene was frequently detected in samples from 10 parking structures in California, with the maximum concentration being 28 μg/m³ (Wilson *et al.*, 1991). Butadiene has also been detected in urban road tunnels during rush hours in Australia (mean concentration 28 μg/m³; Duffy and Nelson, 1996) and Sweden (mean concentrations 17 μg/m³ and 25 μg/m³ in two tunnels; Barrefors, 1996). Butadiene was measured at concentrations ranging from 0.2 to 28 μg/m³ in 96 of 97 5-minute air samples collected from a pumping island at randomly identified self-service filling stations in California (Wilson *et al.*, 1991).

2.3.2.2 Indoor air

Concentrations of butadiene in the air of indoor environments are highly variable and depend largely on individual activities and circumstances, including the use of consumer products (e.g., cigarettes), the infiltration of vehicle exhaust from nearby traffic and possibly from attached garages, and, reportedly, cooking activities involving heated fats and oils. While data are inadequate to determine the relative contributions of each of these potential indoor sources, the highest concentrations of butadiene in indoor air in Canada have generally been detected in indoor environments contaminated with environmental tobacco smoke (ETS).

Butadiene was detected in 45% of indoor air samples in the Windsor Air Quality Study (Bell *et al.*, 1993) but in only 7.5% of outdoor air samples, using the same sampling and analytical methodologies (detection limits 0.08–0.14 μg/m³). A maximum concentration of 1.2 μg/m³ was measured outdoors. Mean concentrations in indoor air from "non-smoking" locations ranged from 0.3 to 1.6 μg/m³, while mean concentrations in indoor air from "smoking" locations ranged from 1.3 to 18.9 μg/m³. A maximum indoor concentration of 36.9 μg/m³ was measured in a bingo hall.

The frequency of detection of butadiene was 75–100% at non-residential indoor sampling sites where ETS was present.

Concentrations of butadiene were measured in 57 randomly chosen homes in Hamilton, Ontario, during 1993 (Hamilton-Wentworth, 1997). In 34 pairs of concurrent 24-hour samples of indoor and outdoor air, butadiene was detected in 38% of the indoor samples but in only 9% of the outdoor samples. Limits of detection ranged from 0.08 to 0.14 μg/m³. A concentration equivalent to one-half the limit of detection was assumed for the concentration of butadiene in samples in which it was not detected for calculation of median and mean concentrations. The mean concentration of butadiene was nine times higher indoors (i.e., 0.27 μg/m³) than outdoors (i.e., 0.03 μg/m³). The maximum concentrations in indoor and outdoor air were 1.5 μg/m³ and 0.13 μg/m³, respectively. Butadiene was detected in 16% of samples from "non-smoking" homes (maximum concentration 1.0 μg/m³) and in 50% of samples from "smoking" homes (maximum concentration 1.2 μg/m³).

Concentrations of butadiene were measured in a multimedia exposure study in several Canadian cities during 1996 and 1997. In the pilot study phase, butadiene was detected in 25% of 24-hour samples of indoor air, but in none of the 44 concurrent 24-hour outdoor air samples (detection limit 0.6 μg/m³) (Cao, 1997). In the second phase of this study, butadiene was detected in 22% of 24-hour samples of indoor air and in only 9% of the 50 concurrent 24-hour outdoor air samples (detection limit 0.9 μg/m³) (Conor Pacific Environmental, 1998). The maximum concentration of butadiene in the indoor air of the 94 residences was 19.2 μg/m³, while the maximum concentration in outdoor air was 2.1 μg/m³. Butadiene was detected in 10% of the indoor air samples from homes (n = 57) where cigarette smoking did not occur (mean concentration <1 μg/m³; censored data) and in 43% of the indoor air samples from homes (n = 37) where cigarette smoking did occur during the sample collection (mean concentration 2.5 μg/m³; censored data).

2.3.2.3 Surface water

No data on concentrations of butadiene in Canadian lake, river, estuarine or marine waters were identified in the literature. Butadiene is being monitored in effluents discharged into the St. Clair River from the butadiene production plant in Sarnia, Ontario. It was detected only twice, at 2 and 5 μg/L, in 2103 composite samples of aqueous effluent taken every 4 hours in 1996 (detection limit 1 μg/L). In daily sampling of effluents from the four individual outfalls (detection limit 1 μg/L in 736 samples and 50 μg/L in 789 samples), butadiene was detected in only three samples, at concentrations of 21, 80 and 130 μg/L (Bayer Inc., 1997).

Using the approaches of Mackay (1991), partition coefficients were calculated for a closed system at steady-state equilibrium at 25°C (Environment Canada, 1998). Under such conditions, it was predicted that for the highest concentration measured in outdoor air in Canada (28 μg/m³), the concentration of butadiene expected in water would be 9.3×10^{-3} μg/L.

2.3.2.4 Groundwater

Butadiene was detected but not quantified in a groundwater plume near a waste site in Quebec where refinery oil residues and a variety of organic chemicals had been dumped (Pakdel *et al.*, 1992).

2.3.2.5 Drinking water

There are no data available concerning the presence of butadiene in drinking water in Canada or elsewhere. In an investigation on whether the use of polybutylene pipe in water distribution systems is likely to result in the contamination of drinking water with butadiene, Cooper (1989) did not detect the substance in water from these types of pipes (no further information was presented in the secondary account [CARB, 1992] of this study).

2.3.2.6 Soil and sediment

No data were identified regarding concentrations of butadiene in soil or sediment. Using the approaches of Mackay (1991), partition coefficients were calculated for a closed system at steady-state equilibrium at 25°C (Environment Canada, 1998). Under such conditions, it was predicted that for the highest concentration measured in outdoor air in Canada (28 µg/m³), the concentrations of butadiene expected in bulk soil and bulk sediment would be 7.5×10^{-6} and 1.5×10^{-5} µg/g (dry weight), respectively.

2.3.2.7 Food

There are no data available concerning the presence or concentrations of butadiene in food in Canada. In the United States, the migration of butadiene from rubber-modified plastic containers to food was investigated by McNeal and Breder (1987). Butadiene was detected in some of the containers, but was generally not detected in the foods (detection limits 1–5 ng/g). Similarly, in the United Kingdom, butadiene was not detected (detection limit 0.2 ng/g) in five brands of soft margarine, although its presence was demonstrated (at concentrations ranging from <5 to 310 ng/g) in the plastic containers (Startin and Gilbert, 1984). Butadiene has been detected in the emissions from heated cooking oils, including Chinese rapeseed, peanut, soybean and canola oils, at levels ranging from 23 to 504 µg/m³ (Pellizzari *et al.*, 1995; Shields *et al.*, 1995).

2.3.2.8 Consumer products

Data on emissions of butadiene from potential indoor sources such as styrene-butadiene rubber were not identified.

Butadiene has been detected in both mainstream smoke and sidestream smoke from cigarettes in Canada and the United States. For 18 brands of Canadian cigarettes, the mean butadiene content ranged from 14.3 to 59.5 µg/cigarette (overall mean concentration 30.0 µg/cigarette) in the mainstream smoke and from 281 to 656 µg/cigarette (overall mean concentration 375 µg/cigarette) in the sidestream smoke, according to "preliminary" data (Labstat, Inc., 1995). The U.S. DHHS (1989) reported that the vapour phase of mainstream smoke of non-filtered cigarettes contained butadiene at levels of 25–40 µg/cigarette. Brunnemann *et al.* (1989) measured butadiene levels ranging from 16 to 75 µg/cigarette in mainstream smoke from seven brands of cigarettes and levels ranging from 205 to 361 µg/cigarette in the sidestream smoke from six types of cigarettes. As discussed in Section 2.3.2.2, the presence of ETS contributes to elevated levels of butadiene in indoor air.

2.4 Effects characterization

2.4.1 Ecotoxicology

Owing to the high vapour pressure, flammable/explosive nature and relatively rapid abiotic and biotic degradation of butadiene, few experimental toxicity data are available, particularly for aquatic organisms. Instead, many of the data have been derived using modelling based on quantitative structure–activity relationships (QSAR). The reliability of the data is dependent on the model used; data reported below were derived using models for non-polar narcotics or organic volatiles. Experimental data for substances chemically or toxicologically related to butadiene can be used to verify the reliability of the modelled data.

There is no experimental information in the literature on the effects of butadiene on aquatic plants or invertebrates. Predicted acute and chronic toxicity data for these groups are presented in Table 2. Toxicity information for the alga *Selenastrum capricornutum* is available for a structurally similar chemical, 1,3-pentadiene, with a 96-hour EC_{50} of 174.6 mg/L for growth rate and 245.8 mg/L for growth inhibition (OECD, 1996). The measured 48-hour EC_{50} for the invertebrate *Daphnia* exposed to 1,3-pentadiene was 221.5 mg/L (OECD, 1996).

No valid aquatic toxicity tests have been carried out in which fish were exposed to

TABLE 2 Acute and chronic environmental toxicity values for butadiene[1]

Test organism	Endpoint[2]	Toxicity value	Reference[2]
Freshwater algae — Acute/chronic			
algae	72-hour EC_{50}	32.6 mg/L	Bol *et al.*, 1993*
green algae	96-hour EC_{50}	27.4 mg/L	Galassi and Vighi, 1981*
Freshwater invertebrate — Acute			
Daphnia sp.	48-hour EC_{50}	44.9 mg/L	Bol *et al.*, 1993*
Daphnia sp.	48-hour EC_{50}	43.8 mg/L	Hermens *et al.*, 1984*
Daphnia sp.	48-hour EC_{50}	24.8 mg/L	IUCLID, 1996
Freshwater invertebrate — Chronic			
Daphnia sp.	21-day NOEC reproduction/growth	9.2 mg/L	Bol *et al.*, 1993*
Daphnia sp.	16-day EC_{50} production	2.2 mg/L	Hermens *et al.*, 1984*
Freshwater fish — Acute			
fathead minnow (*Pimephales promelas*)	96-hour LC_{50}	42.8 mg/L	Bol *et al.*, 1993*
fathead minnow (*Pimephales promelas*)	96-hour LC_{50}	49.8 mg/L	IUCLID, 1996
fathead minnow (*Pimephales promelas*)	96-hour LC_{50}	40.9 mg/L	Veith *et al.*, 1983*
bluegill (*Lepomis macrochirus*)	96-hour LC_{50}	37.8 mg/L	IUCLID, 1996
channel catfish (*Ictalurus punctatus*)	96-hour LC_{50}	21.4 mg/L	IUCLID, 1996
rainbow trout (*Oncorhynchus mykiss*)	96-hour LC_{50}	22.4 mg/L	IUCLID, 1996
Freshwater fish — Chronic			
fathead minnow (*Pimephales promelas*) and zebra fish (*Danio rerio*)	21-day NOEC	4.5 mg/L	Bol *et al.*, 1993*
fish	30-day survival, growth	5.3 mg/L	U.S. EPA, 1991*
fathead minnow (*Pimephales promelas*)	32-day MATC	7.3 mg/L	IUCLID, 1996
Saltwater fish — Acute			
sheepshead minnow (*Cyprinodon variegatus*)	96-hour LC_{50}	9.3 mg/L	Zaroogian *et al.*, 1985*

TABLE 2 (continued)

Test organism	Endpoint	Toxicity value	Reference[2]
Terrestrial plant -- Acute/chronic			
coleus, sorghum, soybean	7-day NOEC[3]	2210 mg/m^3	Heck and Pires, 1962
cotton, cowpea, tomato	7-day LOEC[3]	2210 mg/m^3	Heck and Pires, 1962
cotton, coleus, tomato	21-day NOEC[3]	22.1 mg/m^3	Heck and Pires, 1962
cotton, tomato	21-day LOEC[3]	221 mg/m^3	Heck and Pires, 1962
Soil invertebrate — Chronic			
earthworm (*Eisenia fetida*)	14-day LC_{50}	335 mg/kg (dry soil)	McCarty, 1997

[1] All but terrestrial plant data are estimated using QSAR modelling, assuming a log K_{ow} value of 1.99.

[2] For those references marked with an asterisk, the values presented in the table were calculated using the equations/methods outlined in the references (i.e., the values themselves are not contained in the references).

[3] Experimental observations.

butadiene. A 24-hour Median Tolerance Limit (LC_{50}) of 71.5 mg/L for pinfish (*Lagodon rhomboides*) is frequently quoted for butadiene, but the actual chemical tested was cyano-1,3-butadiene (Daugherty and Garrett, 1951), and so the result is not relevant to this assessment. Predicted acute and chronic toxicity values for freshwater fish are presented in Table 2. Information on toxicity is available for the structurally similar chemicals 1,3-pentadiene and isoprene (3-methyl-1,3-butadiene) (OECD, 1996). For 1,3-pentadiene, the 96-hour LC_{50} for fathead minnow (*Pimephales promelas*) was 139.9 mg/L. For isoprene, the 96-hour LC_{50}s ranged from 42.5 mg/L for bluegill (*Lepomis macrochirus*) to 240 mg/L for the guppy (*Poecilia reticulata*) (OECD, 1996).

The toxicity of butadiene to several species of terrestrial plants has been determined experimentally by Heck and Pires (1962), including effects on growth and development of cotton, cowpea, tomato, coleus, sorghum and soybean. When plants were exposed to butadiene at 2210 mg/m^3 for 7 days, no injury was reported for coleus, sorghum and soybean, and only slight injury was reported in cotton, cowpea and tomato. When exposed for 21 days to butadiene, no injury was seen in coleus, cotton and tomato exposed to 22.1 mg/m^3, and no significant (<5%) injury was seen in cotton and tomato exposed to 221 mg/m^3. The authors summarized the results as 0% injury on exposure to 22.1 mg/m^3 and only slight (<5%) injury on exposure to both 221 and 2210 mg/m^3. The nature of the injury was not stated. The butadiene tested was >99% pure, with impurities including t-butyl catechol, n-butane, butenes and acetylene.

Although there is no information on experimental toxicity for soil invertebrates, modelling was used to estimate a 14-day LC_{50} of 335 mg/kg (dry mass) for earthworm (Table 2) (McCarty, 1997). No information on the effects of butadiene on birds or wild mammals by any route of exposure was identified. Data for laboratory mammals and other organisms pertinent to the human health assessment are presented in Section 2.4.3.

2.4.2 Toxicokinetics and metabolism

The database on the toxicokinetics and metabolism of butadiene in mammals is relatively extensive. The proposed metabolism is outlined in Figure 1, based on the pathways described by

Henderson *et al.* (1993, 1996) and Himmelstein *et al.* (1997). Available data for the pathways most extensively investigated indicate that metabolism is qualitatively similar among the various species studied, although there may be quantitative differences in the amount of butadiene absorbed as well as in metabolic rates and the proportion of metabolites generated. These differences appear to be in concordance with the observed variation in sensitivity to butadiene-induced toxic effects of the few strains of rodent species tested to date, in that mice appear to metabolize a greater proportion of butadiene to active epoxide metabolites than do rats. While less of these metabolites are also formed in samples of human tissues *in vitro* than in those of mice, available data are insufficient to characterize interindividual variability in humans. Although there are known genetic polymorphisms for a number of the enzymes involved in the metabolism of butadiene, information on genotype was not included in most investigations in humans.

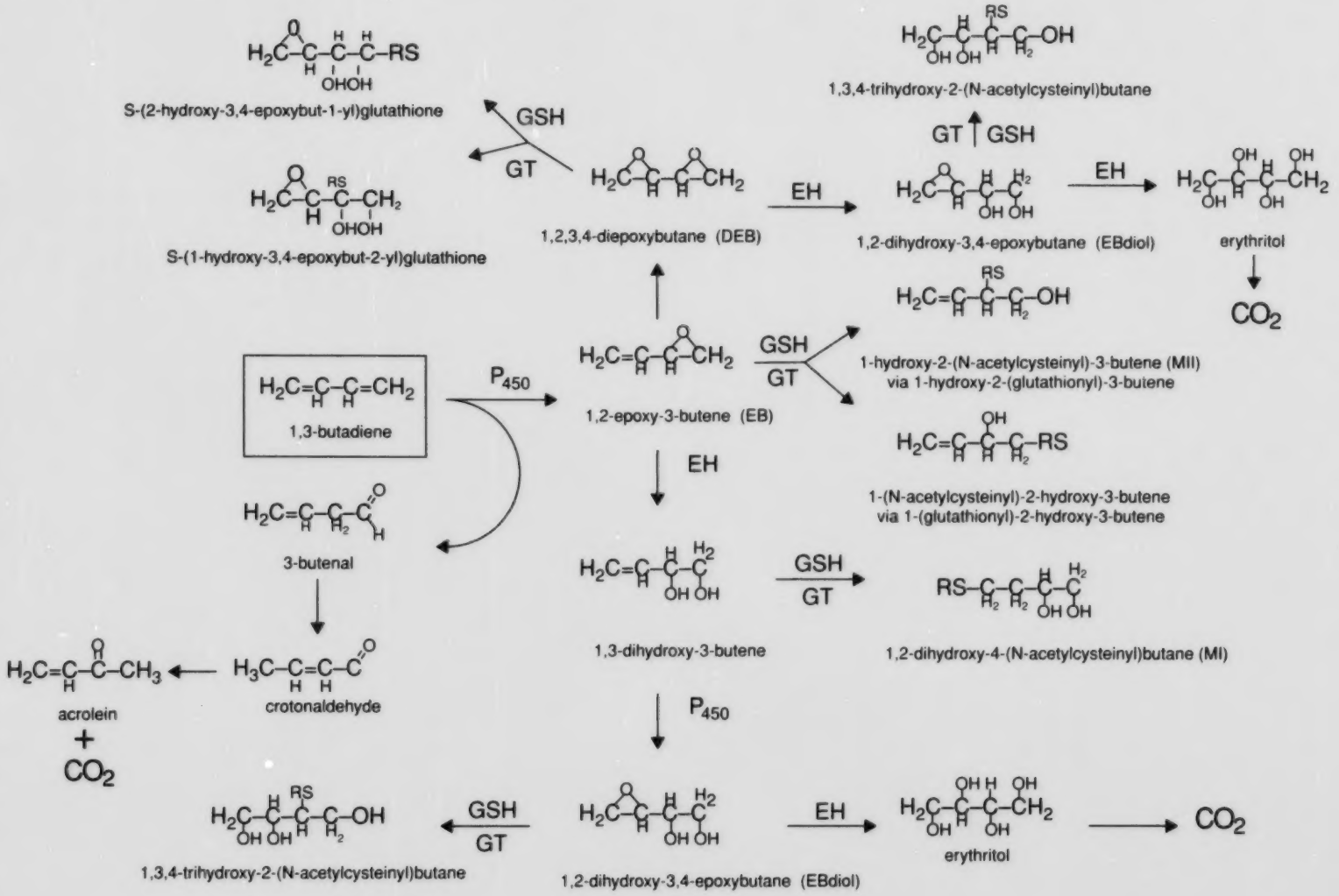
Based on the metabolic pathways described in Figure 1, butadiene is first oxidized via cytochrome P-450 enzymes (primarily P-450 2E1, although other isoforms may also be involved, the relative contribution of which varies between tissues and species) to the monoepoxide 1,2-epoxy-3-butene, or EB, which is subsequently further oxidized via P-450 enzymes to the diepoxide 1,2,3,4-diepoxybutane, or DEB, or hydrolysed via epoxide hydrolase (EH) to butenediol (1,2-dihydroxy-3-butene). The monoepoxide, the diepoxide and the butenediol may all be conjugated with glutathione (GSH) to form mercapturic acids (the latter likely via oxidation to a reactive Michael acceptor), which are eventually eliminated in the urine. Hydrolysis of the diepoxide via epoxide hydrolase or oxidation of the butenediol via cytochrome P-450 will result in the formation of the monoepoxide diol (EBdiol). A small amount of butadiene may be converted to 3-butenal, which is subsequently transformed to crotonaldehyde (about 2–5% of the amount that is oxidized to the monoepoxide in human liver microsomes [Duescher and Elfarra, 1994] or microsomes of kidney, lung or liver of B6C3F$_1$ mice [Sharer *et al.*, 1992]). However, this pathway has not been extensively investigated, nor was crotonaldehyde detected in a sensitive analysis (using nuclear magnetic resonance spectroscopy) of urinary metabolites of rats and mice exposed to ^{13}C-butadiene (Nauhaus *et al.*, 1996).

Metabolism of butadiene and subsequent conversion of EB to DEB may also take place to a more limited degree in the bone marrow (e.g., Maniglier-Poulet *et al.*, 1995) by means other than P-450 oxidation (possibly via myeloperoxidase; Elfarra *et al.*, 1996), based on *in vitro* observations and the detection of the epoxides in the bone marrow of rodents (Thornton-Manning *et al.*, 1995a, 1995b), although this potential pathway has not yet been extensively investigated. EB may also react with both myeloperoxidase and chloride to form a chlorohydrin (1-chloro-2-hydroxy-3-butene) (Duescher and Elfarra, 1992). Metabolites arising from other possible pathways have been identified in the urine of mice exposed to butadiene (including metabolites known to be derived from metabolism of acrolein or acrylic acid) (Nauhaus *et al.*, 1996), but no further research has yet been generated.

There is a substantial amount of evidence from *in vitro* and *in vivo* investigations that B6C3F$_1$ mice oxidize butadiene to the monoepoxide via P-450 (primarily 2E1, although 2A6 and other isoforms may also contribute) in the liver to a greater extent than do Sprague-Dawley rats and humans. Levels of EB in the blood and other tissues of mice were two- to eightfold higher than those in rats exposed to similar levels of butadiene (Bond *et al.*, 1986; Himmelstein *et al.*, 1994, 1995; Bechtold *et al.*, 1995; Thornton-Manning *et al.*, 1997).

Available data also suggest that there are similar species differences in the amount of the diepoxide formed from oxidation of the monoepoxide. Levels of DEB were 40- to 160-fold higher in blood and other tissues of B6C3F$_1$ mice than in Sprague-Dawley rats

FIGURE 1 Proposed metabolism of butadiene in mammals



exposed to the same concentration of butadiene (Thornton-Manning *et al.*, 1995a, 1995b). While concentrations of EB at various sites were similar in male and female rats, levels of DEB were at least fivefold higher in females than in males, which correlates with the greater incidence of tumours in female rats. Although the mammary gland is a target tissue in rats, extended exposure to butadiene at 8000 ppm (17 696 mg/m^3) for 10 days did not result in any accumulation of DEB at this site (Thornton-Manning *et al.*, 1998), which suggests that DEB may not play a significant role in the induction of mammary tumours in rats. Available *in vitro* data in human liver and lung samples suggest that humans also form less of the active metabolites of butadiene than do mice (although somewhat varying results have been reported with respect to the magnitude of the differences between species) (Csanády *et al.*, 1992; Duescher and Elfarra, 1994; Krause and Elfarra, 1997).

Although epoxide metabolites of butadiene are formed to a greater extent in mice than in rats or humans, they are also cleared via glutathione conjugation more rapidly in mice (Kreuzer *et al.*, 1991; Sharer *et al.*, 1992; Boogaard *et al.*, 1996a, 1996b). Conversely, hydrolysis of EB and DEB is greater in humans than in rats, and hydrolysis of EB and DEB in rats is in turn greater than that in mice (Csanády *et al.*, 1992; Krause *et al.*, 1997). In both humans and monkeys, removal of EB via hydrolysis appears to predominate over conjugation with glutathione, based on analysis of urinary metabolites (Sabourin *et al.*, 1992; Bechtold *et al.*, 1994). Although hydrolysis of the epoxide metabolites is generally considered to be a detoxifying mechanism, it may also lead to the formation of the diolepoxide, EBdiol. However, no data were identified on species differences in the formation of EBdiol via metabolism of both epoxide metabolites.

The formation of stable adducts of both the monoepoxide and monoepoxide diol metabolites of butadiene with the N-terminal valine of hemoglobin has been observed in experimental animals and humans exposed to butadiene (Albrecht *et al.*, 1993; Osterman-Golkar *et al.*, 1993, 1996; Neumann *et al.*, 1995; Sorsa *et al.*, 1996b; Tretyakova *et al.*, 1996; Pérez *et al.*, 1997; Swenberg, 1998). Consistent with the greater formation of epoxide metabolites, greater concentrations of hemoglobin–EB adducts were measured in mice than in rats exposed to the same concentration of butadiene. However, levels of hemoglobin–EB adducts in butadiene-exposed workers, although significantly elevated compared with levels in non-exposed workers, were considerably less than would be expected on the basis of results of studies in mice and rats (Osterman-Golkar *et al.*, 1993). Based on observations in rats and humans exposed to butadiene, levels of hemoglobin–EBdiol adducts are substantially greater than levels of hemoglobin–EB adducts (although it is noted that the same adduct can result from binding with DEB). Metabolites of butadiene may also form adducts with DNA (see Sections 2.4.3.4 and 2.4.4.4).

In addition to quantitative interspecies differences in the metabolism of butadiene, there is also evidence that there is significant variation within the human population. Indeed, although available data are inadequate to assess interindividual variation in metabolism, which has been observed in *in vitro* investigations in microsomes from a small number of subjects (Boogaard and Bond, 1996; Krause *et al.*, 1997), there has been significant interindividual variability in the extent of formation of hemoglobin adducts with butadiene metabolites in human populations (Neumann *et al.*, 1995; Osterman-Golkar *et al.*, 1996). Such variability is not unexpected, in view of the complexity of the metabolic pathways involved in the biotransformation of butadiene: i.e., the three principal enzymatic processes that determine the extent of exposure to the putatively toxic epoxide metabolites, namely formation via cytochrome P-450-2E1 and removal via epoxide hydrolase and glutathione conjugation. For example, the inducibility of cytochrome P-450-2E1 by low molecular weight compounds such as ethanol is likely to contribute to interindividual

variability in sensitivity. Moreover, genetic polymorphisms for glutathione-S-transferases and epoxide hydrolase might also contribute to considerable variation in sensitivity. While the influence of genotype for epoxide hydrolase has not been well investigated (although data indicate that hydrolysis of EB predominates over oxidation and glutathione conjugation in humans), interindividual sensitivity to the genetic effects of the epoxide metabolites in *in vitro* studies has been clearly related to genotype for the glutathione-S-transferases (see Section 2.4.4.4).

2.4.3 *Experimental mammals and* in vitro

2.4.3.1 Acute toxicity

Although few data are available, butadiene appears to be of low acute toxicity in experimental animals, with reported LC_{50} values for rats and mice of >100 000 ppm (>221 000 mg/m^3). Lowest LC_{50} values for butadiene are reported for mice, at 117 000 ppm (256 000 mg/m^3) (duration not specified) (Batinka, 1966) and 121 000 ppm (268 000 mg/m^3) (2 hours) (Shugaev, 1969). Exposure to butadiene for 7 hours caused a concentration-dependent depletion of cellular non-protein sulphydryl content of liver, lung or heart in mice, with a Lowest-Observed-Effect Level (LOEL) of 100 ppm (221 mg/m^3) (Deutschmann and Laib, 1989).

2.4.3.2 Short-term and subchronic toxicity

The majority of short-term and subchronic studies were designed as either range-finding studies preliminary to chronic bioassays or investigations of potential mechanisms of action for butadiene-induced cancer and are not adequate for determination of critical effect levels. Effects on body weight were observed in B6C3F$_1$ mice exposed to 625 ppm (1383 mg/m^3) butadiene or more for 2 weeks; no histopathological changes were noted at any concentration at or below 8000 ppm (17 696 mg/m^3) (NTP, 1984).

Hematological effects consistent with megaloblastic anemia and effects on bone marrow, including alterations in stem cell development, have been observed in two strains of mice (B6C3F$_1$ and NIH Swiss) exposed to 1000 or 1250 ppm (2212 or 2765 mg/m^3) butadiene for up to 31 weeks (Irons *et al.*, 1986a, 1986b; Leiderman *et al.*, 1986; Bevan *et al.*, 1996). Other effects, including decreased survival and body weight gain (with males being more sensitive than females), altered organ weights and ovarian or testicular atrophy, have also been observed in B6C3F$_1$ mice exposed subchronically to similar or higher levels of butadiene (NTP, 1984; Bevan *et al.*, 1996). In addition, an increased incidence of a variety of tumours has been observed in B6C3F$_1$ mice exposed to 625 ppm (1383 mg/m^3) butadiene for as little as 13 weeks (NTP, 1993) (see Section 2.4.3.3). Although histopathological changes and hematological effects were reported in early studies in rats exposed to low concentrations (3 or 10 mg/m^3) (Batinka, 1966; Ripp, 1967; Nikiforova *et al.*, 1969), these results were not confirmed in more recent investigations of rats exposed for up to 13 weeks to much higher concentrations (e.g., 17 600 mg/m^3) (e.g., Crouch *et al.*, 1979; Bevan *et al.*, 1996). In view of the limitations of the studies in rats, it is not possible to draw any conclusions regarding species differences in response to subchronic exposure to butadiene.

2.4.3.3 Chronic toxicity and carcinogenicity

The carcinogenic potential of inhaled butadiene has been studied in two strains of mice and one strain of rats. Butadiene was a multi-site carcinogen in all identified long-term experiments, inducing common and rare tumours in mice and rats, although there appear to be marked species and strain differences in sensitivity. In an early bioassay by the National Toxicology Program (NTP, 1984) in which male and female B6C3F$_1$ mice were exposed to 0, 625 or 1250 ppm (0, 1383 or 2765 mg/m^3) butadiene for up to 61 weeks, there were exposure-related increases in the incidences of malignant lymphomas, cardiac hemangiosarcomas

TABLE 3 Incidences of neoplastic lesions in critical carcinogenicity bioassays for butadiene in experimental mammals

Species	Protocol	Results	Comments	Reference
Mice $B6C3F_1$ (70♀ and 70♂ per group; 90♀ and 90♂ per group at the highest concentration)	Mice were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm (0, 13.8, 44.2, 138, 442 or 1383 mg/m^3) butadiene for 6 hours/day, 5 days/week, for 103 weeks. Up to 10 mice of each sex from each group were killed after 9 and 15 months of exposure. Histopathological examination of a comprehensive range of tissues was carried out on mice in the control and 200 and 625 ppm exposure groups killed after 9 months, all mice killed at 15 months except females exposed to 6.25 or 20 ppm, and all mice exposed for 2 years.	**Lymphohematopoietic system** Exposure was associated with the development of malignant lymphomas (particularly lymphocytic lymphomas, which occurred as early as week 23). The incidences were significantly increased in males at 625 ppm ($p < 0.001$) and females at 200 and 625 ppm ($p < 0.001$) (although all incidences in the females were within the range of historical control values [8–44%]). Incidences for the 0, 6.25, 20, 62.5, 200 and 625 ppm groups were: males: 4/50, 2/50, 4/50, 6/50, 2/50 and 51/73, or 8, 4, 8, 12, 4 and 70% females: 6/50, 12/50, 11/50, 7/50, 9/50 and 32/80, or 12, 24, 22, 14, 18 and 40% After poly-3 adjustment for survival, the incidences were: males: 9.0, 4.4, 10.0, 15.3, 7.4 and 97.3% females: 13.1, 27.2, 27.5, 20.2, 40.1 and 85.5% Histiocytic sarcomas were significantly increased in both males ($p < 0.001$) and females ($p = 0.002$) at 200 ppm, and the incidence of these tumours was marginally higher than that in controls in males at 20, 62.5 and 625 ppm ($p = 0.021–0.051$) and females at 625 ppm ($p = 0.038$). Incidences: males: 0/50, 0/50, 4/50, 5/50, 7/50 and 4/73, or 0, 0, 8, 10, 14 and 5% females: 3/50, 2/50, 7/50, 4/50, 7/50 and 4/80, or 6, 4, 14, 8, 14 and 5% Adjusted incidences: males: 0, 0, 10.0, 12.9, 24.3 and 50.7% females: 6.5, 4.4, 17.2, 11.8, 34.0 and 35.5% **Heart** The incidences of cardiac hemangiosarcomas were significantly increased compared with controls in males at 62.5 ppm and above and females at 200 ppm and above. Incidences: males: 0/50, 0/49, 1/50, 5/48, 20/48 and 4/73, or 0, 0, 2, 10, 42 and 5% females: 0/50, 0/50, 0/50, 1/49, 21/50 and 23/80, or 0, 0, 0, 2, 42 and 29% Adjusted incidences: males: 0, 0, 2.6, 13.5, 64.1 and 52.9% females: 0, 0, 0, 3.1, 71.9 and 83.4% **Lungs** There was evidence of increased incidences of alveolar/bronchiolar adenomas or carcinomas compared with controls in males at 62.5 ppm and above ($p < 0.001$) and in females at all concentrations ($p < 0.001–0.004$).	Hemangiosarcomas had not previously been observed in 573 male and 558 female NTP 2-year historical controls. Renal tubule adenomas are rare in this strain, with a range of 0–1% occurrence in NTP 2-year historical controls. Carcinomas of the preputial gland are rare in $B6C3F_1$ mice, with none having been observed in NTP historical controls; similarly, sarcomas of the subcutaneous tissue are uncommon, with the incidence in NTP historical control females being 2/561. Zymbal gland neoplasms have not been observed in NTP historical controls, nor have carcinomas of the small intestine been observed in recent NTP controls.	NTP, 1993

TABLE 3 (continued)

Species	Protocol	Results	Comments	Reference
		Incidences: males: 21/50, 23/50, 19/50, 31/49, 35/50 and 3/73, or 42, 46, 38, 63, 70 and 4% females: 4/50, 15/50, 19/50, 24/50, 25/50 and 22/78, or 8, 30, 38, 48, 50 and 28% Adjusted incidences: males: 47.5, 49.0, 44.9, 74.2, 87.8 and 45.1% females: 8.8, 33.0, 46.5, 61.1, 81.5 and 82.4%	Exposure to butadiene tended to be associated with malignant neoplasms in several organs, whereas tumours at the same sites in controls were generally benign.	
		Forestomach An increased incidence of forestomach tumours (squamous cell papillomas or carcinomas) was observed in males at 200 and 625 ppm ($p < 0.001$) and females at 62.5 ppm and above ($p < 0.001$–0.044). Incidences: males: 1/50, 0/50, 0/50, 1/50, 8/50 and 4/73, or 2, 0, 0, 2, 16 and 5% females: 0/50, 0/50, 3/50, 2/50, 4/50 and 22/80, or 0, 0, 6, 4, 8 and 28% Adjusted incidences: males: 2.3, 0, 0, 2.7, 28.7 and 53.5% females: 0, 0, 7.8, 6.1, 22.5 and 82.6%		
		Ovary Increased incidences of malignant and benign granulosa cell tumours were reported in females exposed to 62.5 ppm and above ($p < 0.001$). Incidences (benign and malignant): 1/49, 0/49, 1/48, 9/50, 8/50 and 6/79, or 2, 0, 2, 18, 16 and 8% Adjusted incidences: 2.3, 0, 2.6, 26.3, 41.1 and 46.5%		
		Harderian gland The incidence of Harderian gland adenomas and carcinomas was increased in both sexes at 62.5 and 200 ppm ($p < 0.001$–0.016). Incidences: males: 6/50, 7/50, 9/50, 20/50, 31/50 and 6/73, or 12, 14, 18, 40, 62 and 8% females: 8/50, 10/50, 7/50, 15/50, 20/50 and 9/80, or 16, 20, 14, 30, 40 and 11% Adjusted incidences: males: 13.5, 15.2, 22.4, 50.8, 80.6 and 64.2% females: 17.5, 22.7, 17.4, 41.2, 70.9 and 58.0%		

TABLE 3 (continued)

Species	Protocol	Results	Comments	Reference
		Mammary gland The incidence of mammary gland tumours (adenoacanthomas, carcinomas and malignant mixed tumours) was increased in females at 62.5 ppm and above ($p < 0.001$–0.004). Most of the neoplasms were carcinomas. Incidences: 0/50, 2/50, 4/50, 12/50, 15/50 and 16/80, or 0, 4, 8, 24, 30 and 20% Adjusted incidences: 0, 4.5, 10.2, 32.6, 56.4 and 66.8% **Liver** In males, the incidence of hepatocellular neoplasms (adenomas and carcinomas) at 200 ppm was significantly greater than that in the controls ($p = 0.03$). The authors also reported increases in hepatocellular neoplasms at 62.5 ppm in females ($p = 0.027$). Incidences (adenomas and carcinomas): males: 21/50, 23/50, 30/50, 25/48, 33/48 and 5/72, or 42, 46, 60, 52, 69 and 7% females: 15/49, 14/49, 15/50, 19/50, 16/50 and 2/80, or 31, 29, 30, 38, 32 and 3% Adjusted incidences (adenomas and carcinomas): males: 44.6, 48.2, 65.2, 61.6, 85.9 and 61.2% females: 33.3, 30.3, 36.4, 51.4, 64.9 and 21.7% **Other tumours** Low incidences of certain uncommon neoplasms also occurred in exposed mice and were considered to be probably related to treatment. These included preputial gland carcinomas (in 5/50 males at 200 ppm, $p < 0.001$) and renal tubule adenomas in both sexes (in 1/50 males at 6.25, 3/48 at 62.5 ppm and 1/49 at 200 ppm, and in 2/50 females at 200 ppm, compared with none in controls). Tumours at other sites that "may be related to exposure" included neurofibrosarcomas or sarcomas of the subcutaneous tissue in females (1/50, 2/50, 3/50, 5/50 and 3/80) and Zymbal gland neoplasia (one adenoma in control males, one adenoma and one carcinoma in females at 625 ppm). The investigators were uncertain whether the low incidence of carcinomas of the small intestine in the treated animals (females: 2 at 6.25 ppm, 1 at 625 ppm, males: 1 each at 6.25, 20 and 62.5 ppm, 2 at 200 ppm, compared with none in controls) was exposure related.		

TABLE 3 (continued)

Species	Protocol	Results	Comments	Reference
Mice B6C3F$_1$ (50♂ per group)	Mice were exposed to butadiene for 6 hours/day, 5 days/week, at concentrations of 200 ppm (442 mg/m^3) for 40 weeks (equivalent to a total exposure of 8000 ppm-weeks), 312 ppm (690 mg/m^3) for 52 weeks (16 000 ppm-weeks) or 625 ppm (1383 mg/m^3) for 13 or 26 weeks (8000 and 16 000 ppm-weeks, respectively). After exposure ceased, mice were kept in control chambers until 103 weeks and evaluated. Histopathological examination of a comprehensive range of tissues was conducted on all mice.	**Lymphohematopoietic system** The incidence of malignant lymphomas (the majority of which were lymphocytic lymphomas) was markedly increased in both groups exposed to 625 ppm ($p < 0.001$) and occurred as early as 23 weeks in the 625 ppm (26 weeks) group. Incidences: 4/50, 8/50, 22/50, 8/50 and 33/50, or 8, 16, 44, 16 and 66%, in the control, 200 ppm (40 weeks), 625 ppm (13 weeks), 312 ppm (52 weeks) and 625 ppm (26 weeks) groups, respectively. Poly-3 adjusted incidences (adjusted for survival): 9.0, 24.1, 56.1, 35.0 and 87.2% The incidence of histiocytic sarcomas was increased in exposed groups ($p < 0.001$–0.036). Incidences: 0/50, 5/50, 2/50, 7/50 and 2/50, or 0, 10, 4, 14 and 4% Adjusted incidences: 0, 16.3, 9.4, 30.6 and 20.4% **Heart** The incidence of cardiac hemangiosarcomas was significantly ($p < 0.001$) increased in all groups, but particularly in mice exposed to 200 or 312 ppm. Incidences: 0/50, 15/50, 7/50, 33/50 and 13/50, or 0, 30, 14, 66 and 26% Adjusted incidences: 0, 47.1, 30.9, 85.2 and 74.5% **Lungs** There was a significant ($p < 0.001$) increase in the incidence of pulmonary neoplasms (alveolar/bronchiolar adenoma or carcinoma) in all exposed groups, particularly when the figures were adjusted to take account of mortality. Incidences: 21/50, 36/50, 28/50, 32/50 and 17/50, or 42, 72, 56, 64 and 34% Adjusted incidences: 47.5, 88.6, 89.5, 88.0 and 87.2% **Liver** The incidence of adenomas or carcinomas in the liver was significantly greater in the 200 ppm group ($p = 0.004$) than in the controls and in all exposed groups when adjusted for survival ($p < 0.01$–0.05). Incidences: 21/50, 33/49, 24/49, 24/50 and 13/50, or 42, 67, 49, 48 and 26% Adjusted incidences: 44.6, 82.4, 80.3, 75.9 and 77.3% **Forestomach** There was a significant ($p < 0.001$) increase in the incidence of squamous cell papillomas or carcinomas of the forestomach in mice exposed to 312 or 625 ppm (both 13 and 26 weeks). Incidences: 1/50, 3/50, 7/50, 9/50 and 10/50, or 2, 6, 14, 18 and 20% Adjusted incidences: 2.3, 10.2, 28.7, 39.2 and 60.7%	Renal tubule adenomas have only rarely been observed in NTP historical controls (1/571). Malignant gliomas and neuroblastomas of the brain rarely develop spontaneously in this strain of mice, with none having been observed in 574 NTP historical controls.	NTP, 1993

TABLE 3 (continued)

Species	Protocol	Results	Comments	Reference
		Harderian gland The incidence of Harderian gland adenomas or carcinomas was significantly ($p < 0.001$) increased compared with controls in all exposed groups. Incidences: 6/50, 27/50, 23/50, 30/50 and 13/50, or 12, 54, 46, 60 and 26% Adjusted incidences: 13.5, 72.1, 82.0, 88.6 and 76.5% **Other tumours** The incidences of kidney adenomas were 0/50, 4/48, 1/50, 3/49 and 1/50 in the control, 200 ppm (40 weeks), 625 ppm (13 weeks), 312 ppm (52 weeks) and 625 ppm (26 weeks) groups, respectively. The incidence of adenomas or carcinomas of the preputial gland was significantly ($p < 0.001$–0.003) increased in the 312 ppm and 625 ppm (13 or 26 weeks) groups, with incidences of 0/50, 1/50, 5/50, 4/50 and 3/50. Malignant gliomas, which were considered to be exposure related, occurred in two mice exposed to 625 ppm for 13 weeks and one exposed to 625 ppm for 26 weeks. Malignant neuroblastomas were observed in two mice exposed to 625 ppm for 13 weeks. The incidence of adenomas or carcinomas of the Zymbal gland was significantly ($p = 0.009$) increased in mice exposed to 625 ppm for 26 weeks (1/50, 1/50, 0/50, 2/50 and 2/50).		
Rats CD (Sprague-Dawley) (110♂ and 110♀ per group)	Rats were exposed to concentrations of 0, 1000 or 8000 ppm (0, 2212 or 17 696 mg/m^3) butadiene for 6 hours/day, 5 days/week. Ten rats per sex per group were killed at 52 weeks. The study was terminated at 105 weeks in females and 111 weeks in males.	**Mammary gland** The incidence of total mammary gland tumours (adenomas or carcinomas) was significantly increased in treated females in both groups ($p \leq 0.01$; incidences 50/100, 79/100, 81/100 in the control, 1000 and 8000 ppm groups, respectively). The positive trend was significant ($p \leq 0.001$). The incidence of multiple mammary gland tumours was also increased in exposed females (8/100, 42/100 and 38/100, or 1.38, 3.70 and 3.33 adenomas per adenoma-bearing rat; latter values from Melnick and Huff, 1992). **Pancreas** The incidence of pancreatic exocrine adenomas was increased at 8000 ppm in males ($p \leq 0.001$) (incidences 3/100, 1/100 and 10/100); exocrine carcinomas also occurred in one male and one female exposed to 8000 ppm, compared with none in other groups.	The authors indicated that the incidence of pancreatic adenomas may be overestimated, due to difficulties in distinguishing between adenomas and hyperplastic foci or nodules in this organ.	Hazleton Laboratories Europe Ltd., 1981a; Owen *et al.*, 1987; Owen and Glaister, 1990

TABLE 3 (continued)

Species	Protocol	Results	Comments	Reference
	A comprehensive range of tissues from rats at the high concentration and control rats and a more limited range from rats at the lower concentration were examined microscopically in animals killed after 52 weeks and at the end of the study.	**Testes** There was a significant ($p \leq 0.01$) exposure-related increase in the incidence of Leydig cell tumours in the testis (incidences 0/100, 3/100 and 8/100). **Thyroid gland** In females, there were significant exposure-related trends for the occurrence of thyroid follicular cell adenomas or carcinomas (0/100, 4/100 and 11/100; $p \leq 0.001$) and uterine sarcomas (1/100, 4/100 and 5/100; $p \leq 0.05$). **Other tumours** The incidence of Zymbal gland carcinomas was significantly increased ($p \leq 0.01$) at the highest concentration in females (0/100, 0/100 and 4/100); in males, the incidences were 0/100, 1/100 and 1/100.	The authors noted that the incidence of testicular tumours was similar to that observed in historical controls at Hazleton Laboratories (i.e., 0–6%). It was stated that the incidences of both uterine sarcomas and Zymbal gland carcinomas were similar to those reported in untreated Sprague-Dawley rats at the study laboratory and may not have been treatment related. The authors also indicated that additional support for the observed increases in tumour incidences not being associated with exposure was provided by the fact that the majority of the Zymbal gland tumours were present in animals killed within 76–90 weeks, while none was observed at the end of the study.	

(an extremely rare tumour in B6C3F$_1$ mice) and lung tumours in both sexes. There were also increased incidences of papillomas or carcinomas of the forestomach, hepatocellular adenomas or carcinomas, ovarian granulosa cell tumours, acinar cell carcinomas of the mammary gland, brain gliomas and Zymbal gland carcinomas (the latter two tumour types have only rarely been observed in this strain of mice at the NTP) in one or both sexes.

Because of the poor survival of mice in the earlier bioassay and to better characterize the exposure–response relationship, the NTP (1993) subsequently exposed B6C3F$_1$ mice to lower concentrations (0, 6.25, 20, 62.5, 200 or 625 ppm [0, 13.8, 44.2, 138, 442 or 1383 mg/m^3]) of butadiene for up to 2 years. Survival was again decreased in most groups (≥20 ppm); at the highest concentration, deaths were principally due to lymphatic lymphomas, which appeared to arise from the thymus and occurred as early as week 23. Non-neoplastic effects observed in exposed mice included a variety of hematological effects, alterations in organ weights, bone marrow atrophy and hyperplasia, atrophy of the thymus, atrophy and angiectasis of the ovaries, uterine atrophy, mineralization of the cardiac endothelium, liver necrosis and olfactory epithelial atrophy. There were significant increases in the incidence of tumours at a variety of sites (incidence data presented in Table 3), including malignant lymphomas (particularly lymphocytic lymphomas), histiocytic sarcomas, cardiac hemangiosarcomas, Harderian gland adenomas and carcinomas, hepatocellular adenomas and carcinomas, alveolar/bronchiolar adenomas and carcinomas, mammary gland adenoacanthomas, carcinomas and malignant mixed tumours, ovarian granulosa cell tumours and forestomach squamous cell papillomas and carcinomas, particularly when the incidences were adjusted for survival. The incidence of alveolar/bronchiolar adenomas or carcinomas was significantly increased in females at all concentrations (i.e., ≥6.25 ppm). Low incidences of uncommon tumours, such as preputial gland carcinomas, Zymbal gland carcinomas in males and renal tubule adenomas in both sexes, were also suspected of being related to exposure. In addition, exposure to butadiene induced malignant tumours at several sites, whereas, in general, tumours at the same sites in control animals were benign.

The NTP also conducted a "stop exposure" experiment in male B6C3F$_1$ mice designed to investigate whether tumour induction was associated with the exposure concentration or the duration of exposure. Animals were exposed to 200 ppm (442 mg/m^3) for 40 weeks or 625 ppm (1383 mg/m^3) for 13 weeks (both equivalent to 8000 ppm-weeks) or to 312 ppm (690 mg/m^3) for 52 weeks or 625 ppm (1383 mg/m^3) for 26 weeks (both equivalent to 16 000 ppm-weeks); all groups were observed for the remainder of the 2-year study. Again, survival was reduced in all exposed mice, largely due to malignant neoplasms, with significant increases in the incidences of lymphocytic lymphomas, histiocytic sarcomas, cardiac hemangiosarcomas, Harderian gland adenomas or carcinomas, hepatocellular adenomas or carcinomas, alveolar/bronchiolar adenomas or carcinomas and squamous cell papillomas or carcinomas of the forestomach (even in mice exposed for only 13 weeks) (incidence data presented in Table 3). In addition, low incidences of several uncommon tumour types (preputial gland carcinomas, Zymbal gland carcinomas, malignant gliomas and neuroblastomas of the brain, Harderian gland carcinomas and renal tubule adenomas) were again observed in one or more of the exposed groups. Concentration may be more important than the duration of exposure in tumour development, as the incidence of malignant lymphomas and squamous cell carcinomas of the forestomach was greater in the groups that had been exposed to 625 ppm for a shorter period than in those exposed to 200 ppm for a longer period (i.e., similar total cumulative exposure) (NTP, 1993).

Acute exposure of B6C3F$_1$ mice for 2 hours to up to 10 000 ppm (22 120 mg/m^3) butadiene, followed by observation for 2 years,

did not induce an increased incidence of tumours at any site (Bucher *et al.*, 1993).

Sensitivity to butadiene-induced thymic lymphoma/leukemia appears to be enhanced by the presence of an endogenous ecotropic retrovirus in $B6C3F_1$ mice, as the incidence of this tumour was greater in male $B6C3F_1$ mice exposed to 1250 ppm (2765 mg/m^3) butadiene for 52 weeks than in male Swiss mice, which do not express an endogenous retrovirus (57% versus 14%). Exposed mice of both strains had elevated incidences of thymic lymphoma/leukemia compared with controls, as did $B6C3F_1$ mice exposed to 1250 ppm for 12 weeks and then observed for an additional 40 weeks, although the MuLV *env* sequence for the retrovirus was detected only in tumours of the $B6C3F_1$ mice. Other tumours reported in the mice exposed for 52 weeks included hemangiosarcomas of the heart (mainly in $B6C3F_1$ mice) and lung tumours. Neoplasms of the glandular and non-glandular stomach were observed in the $B6C3F_1$ mice, whereas adenocarcinomas of the Harderian gland and the thyroglossal duct were observed in the Swiss mice (Irons *et al.*, 1989).

In the only identified long-term bioassay in rats (Hazleton Laboratories Europe Ltd., 1981a; Owen *et al.*, 1987; Owen and Glaister, 1990), male and female Sprague-Dawley rats were exposed to 0, 1000 or 8000 ppm (0, 2212 or 17 696 mg/m^3) butadiene for up to 111 weeks. At 8000 ppm, survival was reduced in both sexes; there were also changes in the relative weights of a number of organs in males at this concentration, along with an increase in the severity of nephrosis of the kidney relative to controls. Relative liver weights were increased in all exposed groups, although there were no exposure-related histopathological effects on the liver. At 8000 ppm, there were increased incidences of follicular cell adenomas and carcinomas of the thyroid gland in females and exocrine adenomas of the pancreas in males (with a carcinoma occurring in a rat of either sex) (incidence data presented in Table 3). In females, the incidence of benign or malignant mammary gland tumours, along with the incidence of animals with multiple mammary gland tumours, was increased at both 1000 and 8000 ppm. The incidence of sarcomas of the uterus and carcinomas of the Zymbal gland increased significantly with level of exposure in females; in addition, a Zymbal gland carcinoma occurred in one male rat at each exposure level. The incidence of Leydig cell tumours of the testes was increased in both groups of exposed males. The investigators suggested that the occurrence of tumours of the testes and Zymbal gland may have been unrelated to exposure, as the incidences observed were reportedly similar to those in other control rats of the same strain in the study laboratory, although it is noted that Zymbal gland tumours were noted in the chronic bioassays in mice discussed above.

Both the mono- and diepoxide metabolites (EB and DEB) have induced local tumours at the site of application in Swiss mice or Sprague-Dawley rats (Van Duuren *et al.*, 1963, 1965, 1966), although available studies are inadequate to evaluate species differences in sensitivity.

It has been hypothesized that the observed greater sensitivity of $B6C3F_1$ mice compared with Sprague-Dawley rats to the induction of thymic lymphoma by butadiene may be related to differences in the potential of EB to affect hematopoietic stem cell differentiation observed in *in vitro* investigations, as suppression of clonogenic response was greater in bone marrow cells from C56BL/6 mice than in those from Sprague-Dawley rats or humans; it was also hypothesized that the subpopulation of progenitor cells affected in mice is not present in humans (Irons *et al.*, 1995).

2.4.3.4 Genotoxicity

The genotoxicity of butadiene has been investigated in a limited range of *in vitro* assays and a more extensive range of *in vivo* tests. Butadiene was mutagenic in *Salmonella typhimurium* strains TA1530 and TA1535 in the presence of metabolic activation with rodent or human S9 preparations (de Meester *et al.*, 1978, 1980; Arce *et al.*, 1990; NTP, 1993; Araki *et al.*,

TABLE 4 Overview of genotoxicity of butadiene and its metabolites in rodents

Endpoint	Mice (strain)	Rats (strain)	Comments	References
BUTADIENE				
Germ cells				
Dominant lethal mutations	+ (CD-1) + ((102/E1 × C3H/E1)F_1)	– (Sprague-Dawley)	results in mice depended upon duration of exposure and timing of exposure relative to mating; rats were exposed to concentrations similar to those that induced effects in mice	Morrissey *et al.*, 1990; Anderson *et al.*, 1993; Adler *et al.*, 1994, 1998; BIBRA International, 1996a, 1996b; Brinkworth *et al.*, 1998
Heritable translocations	+ (C3H/E1)	NT		Adler *et al.*, 1995a, 1998
Other genetic effects on male germ cells (chromosomal aberrations in embryos, DNA damage, sperm head morphology, micronuclei)	+ ((102/E1 × C3H/E1)F_1) + (CD-1) + (B6C3F_1) + (102 × C3H)	NT		Morrissey *et al.*, 1990; Xiao and Tates, 1995; Brinkworth *et al.*, 1998; Pacchierotti *et al.*, 1998a; Tommasi *et al.*, 1998
Somatic cells				
Chromosomal aberrations (bone marrow)	+ (B6C3F_1) + (Swiss)	NT		Irons *et al.*, 1987; Tice *et al.*, 1987; Shelby, 1990; NTP, 1993
Sister chromatid exchanges (bone marrow)	+ (B6C3F_1)	– (Sprague-Dawley)	rats were exposed to much higher concentrations than those that induced effects in mice	Choy *et al.*, 1986; Cunningham *et al.*, 1986; Tice *et al.*, 1987; Arce *et al.*, 1990; Shelby, 1990; NTP, 1993
Micronuclei (bone marrow, blood, spleen)	+ (NMRI) + (B6C3F_1) + (CB6F_1) +((102/E1 × C3H/E1)F_1) + (NIH Swiss)	– (Sprague-Dawley) – (Wistar)	effects in mice were observed at the lowest concentration tested (i.e., 6.25 ppm); male mice appeared to be more sensitive than female mice; rats were exposed to concentrations similar to those that induced effects in mice	Choy *et al.*, 1986; Cunningham *et al.*, 1986; Irons *et al.*, 1986a, 1986b; Tice *et al.*, 1987; Jauhar *et al.*, 1988; Arce *et al.*, 1990; Shelby, 1990; Victorin *et al.*, 1990; NTP, 1993; Przygoda *et al.*, 1993; Adler *et al.*, 1994; Autio *et al.*, 1994; Leavens *et al.*, 1997; Stephanou *et al.*, 1998
hprt mutations (spleen, thymus)	+ ((102/E1 × C3H/E1)F_1) + (B6C3F_1) – (CD-1)	+ (F344)	mice appeared to be more sensitive than rats	Cochrane and Skopek, 1993, 1994b; Tates *et al.*, 1994, 1998; Meng *et al.*, 1998, submitted(b)
Specific locus mutations (mouse spot test)	+ ((102/E1 × C3H/E1)F_1)	NT		Adler *et al.*, 1994

TABLE 4 (continued)

Endpoint	Mice (strain)	Rats (strain)	Comments	References
Transgenic systems (*lacZ, lacI*)	+ ($CD2F_1$ derived) + ($B6C3F_1$ derived)	NT		Recio *et al.*, 1992, 1993, 1996; Sisk *et al.*, 1994; Recio and Meyer, 1995
Unscheduled DNA synthesis (liver)	– ($B6C3F_1$)	– (Sprague-Dawley)		Vincent *et al.*, 1986; Arce *et al.*, 1990
DNA–DNA or DNA–protein crosslinks (liver)	+/– ($B6C3F_1$)	– (Sprague-Dawley)		Jelitto *et al.*, 1989; Ristau *et al.*, 1990; Vangala *et al.*, 1993
DNA binding (liver, lung)	+ ($B6C3F_1$) + ($CB6F_1$)	+ (Wistar) + (Sprague-Dawley) + (F344)	levels of adducts were slightly higher in mice than in rats	Kreiling *et al.*, 1986b; Sorsa *et al.*, 1996b; Koivisto *et al.*, 1997, 1998; Tretyakova *et al.*, 1998a, 1998b
DNA strand breaks and other damage (liver, lung, testes)	+ ($B6C3F_1$) + (NMRI) – (CD-1)	+ (Sprague-Dawley)	results were dependent on analytical method used; there was little quantitative species difference in the degree of strand breakage	Vangala *et al.*, 1993; Walles *et al.*, 1995; Anderson *et al.*, 1997
1,2-EPOXY-3-BUTENE (EB)				
Germ cells				
Dominant lethal mutations	- ($(102/E1 \times C3H/E1)F_1$)	NT		Adler *et al.*, 1997
Other genetic effects on male germ cells (micronuclei)	+ ($F_1(102 \times C3H)$) + (BALB/c)	+ (Lewis) + (Sprague-Dawley)	Lewis rats appeared to be slightly more sensitive than mice	Xiao and Tates, 1995; Lähdetie *et al.*, 1997; Russo *et al.*, 1997
Somatic cells				
Chromosomal aberrations (bone marrow)	+ (C57Bl/6)	NT		Sharief *et al.*, 1986
Sister chromatid exchanges (spleen)	+ (BALB/c)	NT		Stephanou *et al.*, 1997
Micronuclei (spleen, blood, bone marrow)	+ ($F_1(102 \times C3H)$) + (BALB/c) + ($(102/E1 \times C3H/E1)F_1$) + (CD-1)	+ (Lewis) –/+ (Sprague-Dawley)	($F_1(102 \times C3H)$) mice appeared to be more sensitive than Lewis rats; CD-1 mice appeared to be more sensitive than Sprague-Dawley rats	Xiao and Tates, 1995; Adler *et al.*, 1997; Anderson *et al.*, 1997; Lähdetie and Grawé, 1997; Russo *et al.*, 1997; Stephanou *et al.*, 1997
hprt mutations (spleen)	+ ($B6C3F_1$) + ($(102/E1 \times C3H/E1)F_1$)	– (Lewis) – (F344)		Cochrane and Skopek, 1994b; Tates *et al.*, 1998; Meng *et al.*, submitted (a)
Transgenic systems (*lacI*)	– ($B6C3F_1$ derived)	+ (F344 derived)	rats appeared to be more sensitive than mice	Saranko *et al.*, 1998

TABLE 4 (continued)

Endpoint	Mice (strain)	Rats (strain)	Comments	References
DNA strand breaks and other damage (bone marrow, testes)	+ (CD-1)	+/– (Sprague-Dawley)	damage was observed only in bone marrow cells of rats	Anderson *et al.*, 1997
Unscheduled DNA synthesis (testes)	– (CD-1)	NT		Anderson *et al.*, 1997
1,2,3,4-DIEPOXYBUTANE (DEB)				
Germ cells				
Dominant lethal mutations	+ ((102/E1 × C3H/E1)F_1)	NT		Adler *et al.*, 1995b
Other genetic effects on male germ cells (chromosomal aberrations in zygotes, micronuclei)	+ ((C57Bl/Cne × C3H/Cne)F_1) + (F_1(102 × C3H)) + (BALB/c)	+ (Lewis) + (Sprague-Dawley)	Lewis rats appeared to be more sensitive to induction of micronuclei than F_1(102 × C3H) mice	Adler *et al.*, 1995b; Xiao and Tates, 1995; Lähdetie *et al.*, 1997; Russo *et al.*, 1997
Effects on female germ cells (chromosomal aberrations in embryos)	+ ($B6C3F_1$)	NT		Tiveron *et al.*, 1997
Somatic cells				
Chromosomal aberrations (bone marrow)	+ (NMRI)	NT	positive results were also obtained in Chinese hamsters, with NMRI mice being more sensitive than hamsters	Walk *et al.*, 1987
Sister chromatid exchanges (bone marrow, lung, liver)	+ (NMRI) + (Swiss Webster) + (BDF_1)	NT	positive results were also obtained in Chinese hamsters, with NMRI mice being more sensitive than hamsters	Conner *et al.*, 1983; Walk *et al.*, 1987
Micronuclei (spleen, blood, bone marrow)	+ (F_1(102 × C3H)) + (BALB/c) + ((102/E1 × C3H/E1)F_1) + (CD-1)	+ (Lewis) + (Sprague-Dawley)	there was little difference in sensitivity between F_1(102 × C3H) mice and Lewis rats or between CD-1 mice and Sprague-Dawley rats	Adler *et al.*, 1995b; Xiao and Tates, 1995; Anderson *et al.*, 1997; Lähdetie and Grawé, 1997; Russo *et al.*, 1997; Stephanou *et al.*, 1997
hprt mutations (spleen)	+ ($B6C3F_1$) – ((102/E1 × C3H/E1)F_1)	– (Lewis) + (F344)	F344 rats appeared to be more sensitive than $B6C3F_1$ mice	Cochrane and Skopek, 1994b; Tates *et al.*, 1998; Meng *et al.*, submitted (a)
Transgenic systems (*lacI*)	– ($B6C3F_1$ derived)	– (F344 derived)		Recio *et al.*, 1998
DNA binding	+ (ICR)	NT		Mabon *et al.*, 1996

TABLE 4 (continued)

Endpoint	Mice (strain)	Rats (strain)	Comments	References
DNA strand breaks and other damage (bone marrow, testes)	+/– (CD-1)	+/– (Sprague-Dawley)	damage was noted in bone marrow cells only	Anderson *et al.*, 1997
Unscheduled DNA synthesis (testes)	+ (CD-1)	NT		Anderson *et al.*, 1997
1,2-DIHYDROXY-3,4-EPOXYBUTANE (EBdiol)				
Germ cells				
Dominant lethal mutations	– ((102/E1 × C3H/E1)F_1)	NT		Adler *et al.*, 1997
Other genetic effects on male germ cells (micronuclei)	NT	+ (Sprague-Dawley)		Lähdetie *et al.*, 1997
Somatic cells				
Micronuclei (bone marrow)	+ ((102/E1 × C3H/E1)F_1)	+ (Sprague-Dawley)		Adler *et al.*, 1997; Lähdetie and Grawé, 1997

1994), although it was generally inactive in strains TA97, TA98 and TA100 with or without exogenous activation under similar experimental conditions (Victorin and Ståhlberg, 1988; Arce *et al.*, 1990; NTP, 1993). Results of mouse lymphoma assays have been conflicting, with an increased frequency of mutations at the *tk* locus in one study at very high concentrations (i.e., 200 000–800 000 ppm [442 400–1 796 600 mg/m^3]) in the presence of metabolic activation (Sernau *et al.*, 1986), while there was no convincing activity at concentrations of up to 300 000 ppm (663 600 mg/m^3) in another study (although the authors noted that the lack of a positive response may have been due to the low solubility of butadiene in the culture medium; NTP, 1993). Butadiene dissolved in ethanol induced sister chromatid exchanges in cultured mammalian cells (hamsters and humans) (Sasiadek *et al.*, 1991a, 1991b), while *in vitro* exposure to gaseous butadiene did not induce this effect in preparations from rats, mice and humans (Arce *et al.*, 1990; Walles *et al.*, 1995).

An overview of the results of available *in vivo* assays for genotoxicity in germ and somatic cells in mice and rats is presented in Table 4; in general, the data are consistent with species-specific differences in sensitivity to butadiene-induced genetic damage, likely related to the quantitative differences in the formation of active metabolites, although fewer studies have been conducted in rats. Butadiene induced dominant lethal mutations in two strains of mice (CD-1 and (102/E1 × C3H/E1)F_1) following short-term or subchronic exposure of males to concentrations as low as 500 ppm (1106 mg/m^3) for 5 days or 65 ppm (144 mg/m^3) for 4 weeks; however, exposure to 6250 ppm (13 825 mg/m^3) for 6 hours did not induce dominant lethal mutations in CD-1 mice. The results of these studies, which depended upon the timing of mating relative to exposure, suggested that the induction of dominant lethal mutations in mice was likely caused by effects on mature germ cells. In the only similar study in rats identified, there was no evidence of dominant lethal mutations in Sprague-Dawley rats exposed to up to 1250 ppm (2765 mg/m^3) butadiene for 10 weeks.

Short-term exposure to 500 or 1300 ppm (1106 or 2876 mg/m^3) butadiene also induced an exposure-related increase in the incidence of heritable chromosomal translocations in mice; an

increased incidence of chromosomal aberrations was also noted in zygotes of male mice exposed to ≥500 ppm for 5 days. Other butadiene-induced effects observed in male germ cells of mice include sperm head abnormalities, micronuclei in spermatids and DNA damage (strand breaks and alkaline-labile sites). Investigations of these endpoints in rats have not been identified.

Butadiene was consistently genotoxic in somatic cells of several strains of mice, inducing chromosomal aberrations, sister chromatid exchanges and micronuclei in numerous assays; micronuclei have been observed following exposure to concentrations as low as 6.25 ppm (13.8 mg/m^3) butadiene for 13 weeks or 62.5 ppm (138 mg/m^3) for 8 hours. Although only few studies were identified, these effects were not observed in rats exposed to much higher concentrations. However, gene mutations at the *hprt* locus have been induced in both mice and rats, with a four- to sevenfold greater mutagenic potency being determined for mice than for rats. Mutagenic activity was also observed in two transgenic mouse systems and in the mouse spot test. Binding to DNA has been observed in all strains of mice and rats tested; following exposure to butadiene, adducts of both guanine and adenine with the monoepoxide as well as the monoepoxide diol metabolites (EB and EBdiol, respectively) have been observed. The degree of adduct formation was generally similar in the two species or, in some studies, up to twofold greater in mice than in rats. Similarly, there was little quantitative difference in the amount of butadiene-induced single strand breaks in DNA of mice and rats. DNA–DNA and DNA–protein crosslinks were noted in one of two studies in mice, but not in rats exposed to higher concentrations of butadiene.

Metabolites of butadiene have also been mutagenic and clastogenic in numerous *in vitro* and *in vivo* assays (see Table 4 for overview of results of *in vivo* assays). EB, DEB and EBdiol all induced mutations in bacteria and yeast in the absence of exogenous metabolic activation (IARC, 1992; NTP, 1993; Thier *et al.*, 1994; Adler *et al.*, 1997); mutagenic activity was also observed for all three metabolites at two foci in human TK6 lymphoblastoid cells, with DEB being much more potent (Cochrane and Skopek, 1993, 1994a). Conversely, the monoepoxide was much more potent than the diepoxide in the induction of mutations at the *lacI* transgene of fibroblasts obtained from a transgenic rat strain (Saranko and Recio, 1998; Saranko *et al.*, 1998). Both EB and DEB also induced sister chromatid exchanges, chromosomal aberrations and micronuclei in cultured mammalian (including human) cells (IARC, 1992; Xi *et al.*, 1997). Aneuploidy in chromosomes 12 and X was also induced in human lymphocytes, which is notable in view of the fact that aneuploidy in these chromosomes is commonly observed in lymphocytic leukemias (Xi *et al.*, 1997). DEB, but not EB or EBdiol, induced micronuclei in spermatids isolated from rats.

The monoepoxide, diepoxide and monoepoxide diol metabolites all induced micronuclei in germ cells of male mice and rats; in one of these studies, the magnitude of the effect was greater in Lewis rats than in F_1(102 × C3H) mice. There were no consistent patterns in the relative potency of the three metabolites. Chromosomal aberrations in zygotes produced by exposed males and dominant lethal mutations were induced by DEB in mice (strains (C57Bl/Cne × C3H/Cne)F_1 and (102/E1 × C3H/E1)F_1), respectively), whereas EB and EBdiol did not induce dominant lethal mutations. In the only identified investigation of the potential effects on female germ cells, pre-mating exposure of female B6C3F_1 mice to DEB resulted in an increased frequency of chromosomal aberrations in embryos in the absence of ovarian toxicity.

EB, DEB and EBdiol were also genotoxic in somatic cells (bone marrow, peripheral blood, lung and spleen), inducing sister chromatid exchanges, chromosomal aberrations or micronuclei in several strains of mice, rats and hamsters, with little consistent evidence of interspecies differences in sensitivity; in general, the diepoxide was more potent than the

monoepoxide or the monoepoxide diol. Although negative results were obtained in Lewis rats, both EB and DEB induced an increased frequency of *hprt* mutations in B6C3F$_1$ mice and F344 rats, with rats being more sensitive than mice, which may be related to slower clearance in rats. EB induced mutations in the bone marrow of *lacI* transgenic rats, but not in *lacI* transgenic mice; DEB did not induce *lacI* mutations in either species. Meng *et al.* (submitted(a)) suggested that the *hprt* assay is more sensitive to the detection of large deletions induced by DEB than the *lacI* transgene assay. DNA damage (strand breaks or alkaline-labile sites) was caused by EB and DEB in the bone marrow of rats and mice, with DEB being less potent than EB; the only damage observed in haploid testicular cells was in mice exposed to EB. It was suggested that the apparent greater potency of EB compared with DEB may be due to the bifunctional alkylating ability of DEB, subsequent induction of DNA repair and the inability of the alkaline Comet assay employed to measure crosslinks (Anderson *et al.*, 1997).

2.4.3.5 Reproductive and developmental toxicity

Few data on the effects of butadiene on reproductive ability were identified. Exposure to up to 1300 ppm (2876 mg/m^3) for 5 days did not affect the reproductive abilities of male (102/E1 × C3H/E1)F$_1$ mice, based on percentages of successful pairings with unexposed females and unfertilized metaphase I oocytes (Pacchierotti *et al.*, 1998a). Similarly, there were no decreases in mating frequency or pregnancy rate in the dominant lethal studies in mice and rats (Anderson *et al.*, 1993, 1998; BIBRA International, 1996a, 1996b; Brinkworth *et al.*, 1998). Documentation of an earlier study in rats, guinea pigs and rabbits (Carpenter *et al.*, 1944) is too limited for evaluation.

The reproductive organs have consistently been targets of non-neoplastic effects induced by butadiene in subchronic and long-term bioassays in B6C3F$_1$ mice but not in Sprague-Dawley rats, although butadiene-induced tumours of the reproductive organs have been observed in both species. Ovarian atrophy and decreased weight were observed in mice exposed to 1000 ppm (2212 mg/m^3, the only concentration tested) for 13 weeks (Bevan *et al.*, 1996). In the 2-year bioassay conducted by the NTP, there was a significant increase in the incidence of ovarian atrophy in females exposed for up to 2 years to all concentrations tested (i.e., ≥6.25 ppm [≥13.8 mg/m^3]); both the incidence and the severity of this lesion increased with exposure. Ovarian atrophy was also observed at the interim sacrifices at 9 and 15 months at higher concentrations (≥200 and ≥62.5 ppm [≥442 and ≥138 mg/m^3], respectively). Atrophied ovaries characteristically had no evidence of oocytes, follicles or corpora lutea. Angiectasis and germinal epithelial hyperplasia of the ovaries were reported at ≥62.5 and ≥200 ppm (≥138 and ≥442 mg/m^3), respectively, after exposure for 2 years. Uterine atrophy was also noted at concentrations of 200 ppm (442 mg/m^3) or greater. Survival was decreased at ≥20 ppm (≥44.2 mg/m^3), principally due to neoplastic lesions at several sites, including the ovaries (Melnick *et al.*, 1990; NTP, 1993).

Effects on the testes, including reduced weight, degeneration or atrophy, were observed in B6C3F$_1$ mice exposed to concentrations at or above 200 ppm (442 mg/m^3) for 2 years or to higher levels for shorter durations (NTP, 1993; Bevan *et al.*, 1996). Cytotoxic effects on differentiating spermatogonia were noted in (102/E1 × C3H/E1)F$_1$ mice 21 days after exposure to ≥130 ppm (≥288 mg/m^3) for 5 days; a decrease in elongated spermatids was noted in mice exposed to 1300 ppm (2876 mg/m^3) (Pacchierotti *et al.*, 1998a).

No non-neoplastic effects were noted in the reproductive organs of male or female Sprague-Dawley rats exposed to up to 8000 ppm (17 696 mg/m^3) butadiene for 2 years (Hazleton Laboratories Europe Ltd., 1981a; Owen *et al.*, 1987).

Both the mono- and diepoxide metabolites of butadiene induced ovarian toxicity (depletion of small and growing follicles) and

alkylation with macromolecules in the ovary in B6C3F$_1$ mice repeatedly exposed via intraperitoneal injection. In contrast, effects in the ovary in Sprague-Dawley rats were observed only in rats exposed to the diepoxide at doses higher than those that were active in the mice (Doerr *et al.*, 1996). Since the results of structure–activity studies with 4-vinylcyclohexene and several of its analogues, butadiene monoepoxide and diepoxide, epoxybutane and isoprene, indicated that compounds that form only monoepoxides do not induce ovarian toxicity (Doerr *et al.*, 1995), it appears that conversion to the bifunctional diepoxide may be required for the induction of these effects. A single intraperitoneal injection of DEB reduced various testicular cell populations and induced morphological changes in the epithelium of the seminiferous tubules in male B6C3F$_1$ mice (Spano *et al.*, 1996).

Few studies on the potential for butadiene to induce developmental effects have been identified. There was no evidence of teratogenicity following exposure of pregnant CD-1 mice to up to 1000 ppm (2212 mg/m^3) butadiene on days 6 through 15 of gestation, although maternal toxicity (decreased body weight gain) and fetal toxicity (reduced fetal weight and skeletal abnormalities) occurred at 200 ppm (442 mg/m^3) and above, and there was a slight reduction in male fetal body weight at 40 ppm (88 mg/m^3) of questionable biological significance (Hackett *et al.*, 1987b; Morrissey *et al.*, 1990). In Sprague-Dawley rats exposed to 8000 ppm (17 696 mg/m^3) butadiene on days 6 through 15 of gestation, there was an increased incidence of "major" abnormalities of the skull, spine, sternum, long bones and ribs. Abnormalities, believed to be associated with retarded embryonic growth, were observed at 200 and 1000 ppm (442 and 2212 mg/m^3). Maternal toxicity (decreased body weight gain or loss of body weight) was observed in all exposed groups (Hazleton Laboratories Europe Ltd., 1981b, 1982). However, there was no evidence of developmental toxicity in Sprague-Dawley rats exposed to up to 1000 ppm (2212 mg/m^3) butadiene, also on days 6 through 15 of gestation, although maternal toxicity (decreased body weight gain) was noted at the highest concentration (Hackett *et al.*, 1987a; Morrissey *et al.*, 1990).

Although evidence of male-mediated teratogenicity was observed when male CD-1 mice exposed to 12.5 ppm (27.7 mg/m^3) butadiene for 10 weeks were mated with unexposed females (Anderson *et al.*, 1993), there was no increase in malformations when the study was repeated at 12.5 and 125 ppm (27.7 and 277 mg/m^3) (Brinkworth *et al.*, 1998). The authors suggested that the discrepant results may be a function of the statistical significance in the first study being due to the lack of abnormalities in controls (compared with 2.5% in exposed), whereas a low incidence was noted in exposed and control mice in the follow-up study. Similarly, there were no significant increases in fetal abnormalities in CD-1 mice following paternal exposure to up to 6250 ppm (13 825 mg/m^3) butadiene for 6 hours (Anderson *et al.*, 1993) or concentrations up to 130 ppm (288 mg/m^3) for 4 weeks (BIBRA International, 1996a), although it was noted in the latter study that some females may have been sacrificed too early for detection of abnormalities. There was no evidence of male-mediated teratogenicity in offspring of male Sprague-Dawley rats exposed for 10 weeks to concentrations as high as 1250 ppm (2765 mg/m^3) butadiene and then mated with unexposed females (BIBRA International, 1996b).

2.4.3.6 Immunotoxicity

Although the hematopoietic system is a target of butadiene-induced toxicity, no effects on immune system function of biological significance were observed in the only relevant study identified in which B6C3F$_1$ mice were exposed to 1250 ppm (2765 mg/m^3) butadiene for up to 24 weeks, although there were depressions in cellularity and plaque-forming cells as well as histopathological changes in the spleen (Thurmond *et al.*, 1986).

TABLE 5 Summary of measures of risk for cancers of the lymphohematopoietic system in populations occupationally exposed to butadiene

Cohort description	Cohort size	Number of cases	Exposure	Risk measure[1] (95% CI)	Comments	Reference
Leukemia						
Styrene-butadiene rubber workers	15 649	48		SMR = 131 (97–174)	SMR was significant in some subgroups; increasing trend in RRs remained when adjusted for styrene	Delzell *et al.*, 1995
		7	0 ppm-years	RR = 1.0		
		14	>0–19 ppm-years	RR = 1.4 (0.4–4.8)		
		18	20–99 ppm-years	RR = 2.3 (0.7–7.9)		
		7	100–199 ppm-years	RR = 2.6 (0.7–10.0)		
		5	≥200 ppm-years	RR = 4.2 (1.0–17.4)		
Butadiene production workers	2795	13		SMR = 113 (60–193)	no association between qualitative measure of cumulative exposure and leukemia risk	Divine and Hartman, 1996
	996	3	low	SMR = 67 (13–195)		
	1874	11	varied	SMR = 154 (77–275)		
Butadiene production workers	364	2		SMR = 123 (15–444)		E.M. Ward *et al.*, 1995, 1996
Lymphosarcoma						
Styrene-butadiene rubber workers	15 649	11		SMR = 80 (40–144)	SMRs were increased for maintenance workers (O = 8; SMR = 192; 95% CI = 83–379) and labourers (O = 3; SMR = 123; 95% CI = 25–359), but not in production or laboratory workers	Delzell *et al.*, 1995

TABLE 5 (continued)

Cohort description	Cohort size	Number of cases	Exposure	Risk measure[1] (95% CI)	Comments	Reference
Butadiene production workers	2795	9		SMR = 191 (87–364)	no association with duration of employment, based on only two and one cases in the two higher categories	Divine and Hartman, 1996
		0	background	SMR = 0 (0–591)		
		2	low	SMR = 109 (12–395)		
		7	varied	SMR = 249 (100–513)		
Butadiene production workers	364	4		SMR = 577 (157–1480)	trend with duration of employment when dichotomized at 2 years	E.M. Ward *et al.*, 1995, 1996
		1	<2 years	SMR = 303		
		3	≥2 years	SMR = 827 ($p < 0.05$)		

[1] SMR = O/E × 100.

2.4.4 *Humans*

2.4.4.1 Clinical studies

The only identified clinical study (Carpenter *et al.*, 1944) is inadequate for evaluation of the potential effects of butadiene in humans.

2.4.4.2 Carcinogenicity

The carcinogenicity of butadiene has been investigated in several populations of workers occupationally exposed during its manufacture or use. Although most of these studies are limited by the paucity of historical monitoring data, there is evidence that occupational exposure to butadiene in the styrene-butadiene rubber industry is associated with excess mortality due to leukemia and weaker evidence of an association with lymphosarcoma[1] in butadiene monomer production workers. A summary of the measures of risk for lymphohematopoietic cancers is presented in Table 5.

In the most recent update of the largest of the cohorts of male monomer workers (n = 2795) at the Port Neches butadiene production facility in Texas (Divine and Hartman, 1996), mortality due to lymphohematopoietic cancer was significantly elevated (standardized mortality ratio [SMR][2] = 147; 95% confidence interval [CI] = 106–198), due largely to a non-significant increase in the number of deaths due to lymphosarcoma and reticulosarcoma (SMR = 191, 95% CI = 87–364), based on nine cases. However, there was no association with duration of employment (SMRs of 261, 182 and 79, for <5, 5–19 and ≥20 years of employment, respectively, based on six, two and one cases). The greatest excess was observed in men first employed during the Second World War (observed cases [O] = 7; SMR = 241; 95% CI = 97–497), during which the greatest concentrations of butadiene likely occurred, although no data were presented (Divine *et al.*, 1993). When the entire cohort was subdivided on the basis of a qualitative measure of exposure (based on job history information, discussions and reviews of

[1] The terminology for cancers of the lymphohematopoietic system is that used by authors of the individual study accounts.

[2] SMRs are presented here in the format used by the authors of the studies; i.e., SMR = observed/expected or SMR = observed/expected × 100.

classifications with long-term employees and recent industrial hygiene surveys), the SMR for lymphosarcoma and reticulosarcoma was greatest (SMR = 249; 95% CI = 100–513) in those whose jobs involved "varied" exposure to butadiene (i.e., the group with potentially the greatest exposure), although the number of cases in each subgroup was small, and only one of the seven cases in this group had been exposed for more than 10 years. There was also a non-significant increase in mortality due to leukemia in the varied exposure group (SMR = 154; 95% CI = 77–275; based on 11 cases, 10 of which had been employed less than 10 years), although there was no such increase in the total cohort (SMR = 113; 95% CI = 60–193). However, there was no association between estimates of cumulative exposure and any form of lymphohematopoietic cancer.

Mortality due to lymphosarcoma and reticulosarcoma was significantly increased in a smaller cohort study of 364 workers who had ever been employed in butadiene production at two Union Carbide plants in West Virginia, based on four cases (SMR = 5.77; 95% CI = 1.57–14.8). The risk was greater in those employed for 2 years or more than in those employed less than 2 years (SMRs of 8.27 and 3.03, respectively), although the number of cases in each category was very small. No significant increase in mortality due to leukemia or aleukemia was observed (observed/expected = 2/1.62). However, no monitoring data were available to assess exposure of individuals in the cohort (E.M. Ward *et al.*, 1995, 1996). In the only other study of monomer production workers, there were no deaths due to cancer of the lymphohematopoietic system; however, the size of the cohort (n = 614) and duration of follow-up were insufficient to detect excess risks of lymphohematopoietic cancer of less than fivefold (Cowles *et al.*, 1994).

Several studies have been conducted in workers employed in the manufacture of synthetic rubber in North America who were exposed to butadiene as well as to styrene and other substances. The largest and most comprehensive study to date involved 15 649 workers employed at eight styrene-butadiene rubber manufacturing facilities in North America (Delzell *et al.*, 1995). The results of this study are emphasized here and considered to supplant those of earlier investigations, as there is considerable overlap in the cohort population with the earlier studies (i.e., 14 869 of these subjects had been employed at one of the two plants studied previously by Meinhardt *et al.* [1982] or at seven of the eight plants investigated by Matanoski *et al.* [1990, 1993] and Santos-Burgoa *et al.* [1992], although they had been employed for different time periods [Delzell *et al.* (1995) included several more years of follow-up] and selected using different inclusion criteria).[3] Estimates of cumulative exposure and peak exposure frequency were derived for workers from six of the eight plants based on complete work histories for 97% of these employees, information on processes and plant conditions based on available records, walk-through surveys and interviews with long-term employees, plant engineers and managers and were compared with monitoring data from surveys conducted from the late 1970s onward.

There was an increase in mortality due to leukemia, which was of borderline statistical significance, in the overall cohort, based on 48 cases (SMR = 131; 95% CI = 97–174); this excess was concentrated in workers who had had 10 or more years of employment and 20 or more years since date of hire (O = 29; SMR = 201; 95% CI = 134–288). Similarly, there was a significant increase in mortality due to leukemia in "ever hourly" workers (i.e., workers who had ever been paid on an hourly basis), whose jobs were most likely to have involved exposure to butadiene (O = 45; SMR = 143; 95%

[3] It is not possible to determine, with any certainty, the size of the population in these earlier studies that was not subsumed in the later investigation by Delzell *et al.* (1995). One of the small plants of approximately 600 workers included in the Matanoski *et al.* (1990, 1993) cohort was not examined by Delzell *et al.* (1995).

CI = 104–191), which was again concentrated in workers with longer duration of employment and time since hire and was greater in black workers than in white workers in this subgroup. The SMRs for leukemia also increased with duration of employment for ever hourly workers. When examined by type of employment, the number of deaths due to leukemia was significantly increased in production workers (O = 22; SMR = 159; 95% CI = 100–241), labourers (O = 16; SMR = 195; 95% CI = 112–317; concentrated among black workers), laboratory workers (O = 12; SMR = 462; 95% CI = 238–806) and black workers in other operations (O = 3; SMR = 680; 95% CI = 137–1986); no significant increases were observed in maintenance workers (O = 13; SMR = 107; 95% CI = 57–184). As well, the SMRs for lymphosarcoma were non-significantly increased for maintenance workers (O = 8; SMR = 192; 95% CI = 83–379) and labourers (O = 3; SMR = 123; 95% CI = 25–359), while there was no increase in mortality due to lymphosarcoma in production workers or laboratory workers. When individual plants were considered separately, there were non-statistically significant increases in mortality due to leukemia at most (but not all) plants (SMRs ranged from 72 to 780, excluding groups in which zero cases were observed and less than one case was expected); numbers of observed cases of lymphosarcoma were too low to permit meaningful conclusions with respect to mortality at individual plants.

In regression analyses, mortality due to leukemia was observed to increase with cumulative exposure to butadiene, as relative risk (RR) values for exposure categories of 0, >0–19, 20–99, 100–199 and ≥200 ppm-years were 1.0, 1.4, 2.3, 2.6 and 4.2, respectively (for cases in which leukemia was considered the underlying cause of death). There was only limited evidence of an association with cumulative exposure to peak levels of butadiene. The authors also investigated the potential influence of exposure to styrene or benzene on mortality and determined that the trend for increased risk with increased cumulative exposure to styrene was less pronounced, while exposure to benzene was considered to be too infrequent (few subjects were exposed) and too low to be a confounding factor. There was no association between cumulative exposure to butadiene and non-Hodgkin's lymphoma in regression analyses.

Based on the results of this study, Delzell *et al.* (1995) concluded that there was a relationship between employment in the styrene-butadiene industry and leukemia, with the increased risk of leukemia being most strongly associated with exposure to butadiene or to butadiene and styrene in combination (although the association with butadiene remained after controlling for exposure to styrene). Data were insufficient to draw any firm conclusions with respect to an association with any specific form of leukemia.

In a subsequent study in which Delzell *et al.* (1996) attempted to better define exposure of this cohort to peak levels of butadiene, the RR for leukemia increased with increasing average annual number of peaks to which workers were exposed (RRs of 1.0, 2.3 and 3.1) as well as again with cumulative exposure to butadiene (RRs of 1.0, 1.1, 2.0, 2.4 and 4.6). Risk of leukemia also increased with duration of employment in areas in which there was "definite" exposure to peaks (RRs of 1.0, 2.3 and 2.7) and in areas for which elevated SMRs had been noted in the previous analyses (RRs of 1.0, 1.9 and 3.1). The authors noted that it was not possible to distinguish between the roles of estimated peak or cumulative exposure.

The estimates of exposure were further refined for workers at one of the plants included in the investigation by Delzell *et al.* (1995) through more extensive research of historical conditions (Macaluso *et al.*, 1997). Although there was little change in classification of various workers as exposed or non-exposed, the revised estimates of cumulative exposure to butadiene for many job groups were generally greater (two- to threefold) than the original; the most substantial increase (by an order of magnitude) was

determined for tasks among unskilled labourers during the 1950s and 1960s. It was not indicated in the report if the rank order of the cumulative exposure estimates differed (although it is likely that it did not; Gerin and Siemiatycki, 1998). There was little change in estimated exposure to peak levels of butadiene or in cumulative exposure to styrene. These revised exposure estimates have not yet been incorporated into cancer mortality analyses.

Sathiakumar *et al.* (1998) re-examined the mortality of this cohort based on currently accepted terminology for lymphohematopoietic cancers (other than leukemia). There were no significant increases in deaths in the overall cohort due to non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma or cancers of other lymphatic tissue, nor were there any associations between mortality due to these causes and duration of exposure and year of hire. Similarly, mortality due to these causes was not associated with any process group; however, the authors noted that an association for non-Hodgkin's lymphoma may be obscured by the possibility that some cases of non-Hodgkin's lymphoma had transformed to leukemia, with the latter form of cancer being recorded on the death certificate.

An association between exposure to butadiene and leukemia, as well as Hodgkin's disease, was also observed in a recent, independently conducted nested case–control study of 58 cases of lymphohematopoietic cancers from a cohort of styrene-butadiene rubber workers (from many of the same plants investigated by Delzell *et al.* [1995]), in which exposure was estimated based on analyses of monitoring data obtained in the last 15–20 years of operation (Manatoski *et al.*, 1997).

Irons and Pyatt (1998) noted the concordance between the potential for exposure to dimethyldithiocarbamate (a "stopper" used in the polymerization process in the styrene-butadiene rubber industry) and mortality due to leukemia in various processes. However, although the biological plausibility of a potential association is recognized (since dimethyldithiocarbamate is a potent inhibitor of clonogenic response in human CD34+ bone marrow cells), it is not possible to draw any conclusion concerning its potential role in the observed increases in leukemia mortality at this time in view of the current lack of quantification of exposure levels of this substance in the plants examined and the absence of data on its leukemogenic potential.

Other epidemiological studies in populations occupationally exposed to butadiene have been identified in the literature (e.g., McMicheal *et al.*, 1974, 1976; Andjelkovich *et al.*, 1976, 1977; Linet *et al.*, 1987; Siemiatycki, 1991; Bond *et al.*, 1992; Downs *et al.*, 1993). However, due to limitations of these studies (including small numbers of observed and expected cases of lymphohematopoietic cancer or lack of exposure characterization), they contribute little to evaluation of the association between exposure to butadiene and these forms of cancer.

Significantly increased mortality due to cancers other than those of the lymphohematopoietic system has not been consistently observed in these studies.

2.4.4.3 Non-neoplastic effects

Mortality due to all causes was similar to or significantly lower than that expected in all of the major cohorts of workers potentially exposed to butadiene. Although increased mortality due to arteriosclerotic or ischemic heart disease or circulatory disease in general has been observed in some subgroups of workers in some of these cohorts (McMichael *et al.*, 1974, 1976; Matanoski *et al.*, 1990; Delzell *et al.*, 1995), the potential association with exposure to butadiene has not been extensively investigated.

There were no differences in morbidity or various hematological parameters between 438 workers exposed to mean concentrations of butadiene ranging up to 10 ppm (22 mg/m^3) (with a maximum time-weighted average concentration of 143 ppm [316 mg/m^3]) and 2600 unexposed

workers at a butadiene production facility in Texas (Cowles *et al.*, 1994). However, Checkoway and Williams (1982) observed changes in hematological parameters consistent with bone marrow depression in eight workers exposed to high concentrations of butadiene (up to about 53 ppm [117 mg/m^3]) when compared with values for 145 workers exposed to much lower levels (i.e., <1 ppm [<2.2 mg/m^3]).

2.4.4.4 Genotoxicity

The potential genotoxicity of butadiene has recently been investigated in several studies of groups of workers exposed in the production of butadiene, styrene-butadiene rubber or polybutadiene rubber. Although the data available to date are not completely consistent, they indicate that there is some evidence that exposure to butadiene induces genetic effects in occupationally exposed populations and that sensitivity to the induction of these effects is related to genetic polymorphism for enzymes involved in the metabolism of butadiene, most notably those within the glutathione-S-transferase class. The results of several *in vitro* studies in human lymphocytes have demonstrated that sensitivity to DEB-induced sister chromatid exchanges and micronuclei is associated with the presence or absence of homozygous deletion of the *GSTT1* gene, which codes for GSTθ (Kelsey *et al.*, 1995; Norppa *et al.*, 1995; Wiencke *et al.*, 1995; Landi *et al.*, 1996; Pelin *et al.*, 1996; Vlachodimitropoulos *et al.*, 1997). Similarly, sensitivity to sister chromatid exchanges induced by EB appears to be related to genotype for *GSTM1*, which codes for GSTμ (Wiencke and Kelsey, 1993; Uuskula *et al.*, 1995), and possibly also *GSTT1* genotype in *GSTM1*-null individuals (Bernardini *et al.*, 1998). However, there were no differences in sensitivity to sister chromatid exchanges induced by EBdiol in individuals with and without deletions for *GSTT1* or *GSTM1* (Bernardini *et al.*, 1996).

Although no increased frequencies of sister chromatid exchanges, chromosomal aberrations or micronuclei were observed in earlier studies in butadiene production workers in Portugal and the Czech Republic compared with controls (Sorsa *et al.*, 1994, 1996b), positive results for chromosomal aberrations and sister chromatid exchanges were obtained in the most recent study of the Czech workers (Tates *et al.*, 1996; Srám *et al.*, 1998). When genotype was considered, there was a significant increase in the frequency of chromosomal aberrations in both exposed and control subjects from both plants who were deficient for the *GSTT1* gene (Sorsa *et al.*, 1996a).

An increased frequency of *hprt*$^-$ mutants in peripheral blood lymphocytes has been observed in two studies of exposed workers at a butadiene production facility in Texas (Legator *et al.*, 1993; Ward *et al.*, 1994; Au *et al.*, 1995) and in preliminary results of a study of styrene-butadiene rubber workers from the same region (J.B. Ward *et al.*, 1996; Ward, 1997a). Although analyses by genotype are not yet available, it was noted that the highest frequency of *hprt*$^-$ variants occurred in an individual who was *GSTT1* null. In contrast to the observations in the Texan plants, however, no increase in *hprt*$^-$ mutant frequency was observed in workers exposed to similar levels of butadiene at the monomer plant in the Czech Republic (Tates *et al.*, 1996) or in a population of polybutadiene rubber workers in China (Hayes *et al.*, 1996) (no information on genotype was presented). These investigations involved different analytical methodologies (autoradiographic versus clonal assays), which may account for the discordance in the results; in addition, differences in occupational scenarios, exposure levels, age, smoking habits or other lifestyle factors may have contributed to the discrepancy. Current ongoing research (including genotyping) may explain the differences in the results.

Decreased DNA repair ability was also observed in peripheral blood lymphocytes of exposed workers at the monomer production and styrene-butadiene rubber facilities in Texas in both a γ-radiation challenge assay and a CAT-Host Cell Reactivation assay (Hallberg *et al.*, 1997; Ward, 1997b). However, the difference between exposed and "unexposed" monomer workers in the response to the challenge assay was no longer

significant after ambient levels in the plant were reduced. Similarly, the effect on DNA repair ability in styrene-butadiene rubber workers was less when only non-smokers were considered.

The detection of alkylated DNA (the same adduct as detected in the liver of mice and rats exposed to butadiene; Jelitto *et al.*, 1989; Koivisto *et al.*, 1997) in the urine of an exposed worker (Peltonen *et al.*, 1993) also provides some evidence of the interaction of butadiene or its metabolites with genetic material in humans.

2.4.5 *Abiotic atmospheric effects*

The potential for butadiene to contribute to the depletion of stratospheric ozone, to global warming or to formation of ground-level ozone was examined.

Since butadiene is not a halogenated compound, its Ozone Depletion Potential (ODP) is 0, and it will therefore not contribute to the depletion of stratospheric ozone (Bunce, 1996).

Gases involved in global warming strongly absorb infrared radiation of wavelengths between 7 and 13 μm, enabling them to trap and re-radiate the Earth's thermal radiation (Wang *et al.*, 1976; Ramanathan *et al.*, 1985). Worst-case calculations were made to determine if butadiene has the potential to contribute to climate change (Bunce, 1996), assuming it has the same infrared absorption strength as the reference compound CFC-11. The Global Warming Potential (GWP) was calculated to be 2.5×10^{-5} (relative to the reference compound CFC-11, where GWP for CFC-11 = 1), based on the following formula:

$$GWP = (t_{butadiene}/t_{CFC-11}) \times (M_{CFC-11}/M_{butadiene}) \times (S_{butadiene}/S_{CFC-11})$$

where:

- $t_{butadiene}$ = lifetime of butadiene = $5.9 \cdot 10^{-4}$ years
- t_{CFC-11} = lifetime of CFC-11 = 60 years
- M_{CFC-11} = molecular weight of CFC-11 = 137.5 g/mol
- $M_{butadiene}$ = molecular weight of butadiene = 54 g/mol
- $S_{butadiene}$ = infrared absorption strength of butadiene = 2389 cm^{-2} atm^{-1} (worst case)
- S_{CFC-11} = infrared absorption strength of CFC-11 = 2389 cm^{-2} atm^{-1}

Since this estimate for the GWP is much less than 1% of that of the reference compound, butadiene is not considered to be involved in climate change (Bunce, 1996).

The contribution of VOCs to the formation of ground-level ozone and the resulting contribution to smog formation is a complex process and has been studied extensively. The terms reactivity, incremental reactivity and photochemical ozone formation potential denote the ability of an organic compound in the atmosphere to influence the formation of ozone (Paraskevopoulos *et al.*, 1995). Estimates of reactivity of a substance depend on the definition and method of calculation of the reactivity, the VOC/NOx ratio, the age of the air mass, the chemical mechanisms in the model, the chemical composition of the hydrocarbon mixture into which the VOC is emitted, the geographical and meteorological conditions of the airshed of interest (including temperature and intensity and quality of light), and the extent of dilution (Paraskevopoulos *et al.*, 1995).

The Photochemical Ozone Creation Potential (POCP) is one of the simpler indices of the potential contribution of an organic compound to the formation of ground-level ozone, based on the rate of reaction of the substance with the hydroxyl radical relative to ethene (CEU, 1995). Ethene, a chemical that is considered to be important in ozone formation, has an assigned POCP value of 100. The POCP for butadiene was estimated to be 407 relative to ethene, using the following formula (Bunce, 1996):

$$POCP = (k_{butadiene}/k_{ethene}) \times (M_{ethene}/M_{butadiene}) \times 100$$

where:

- $k_{butadiene}$ is the rate constant for the reaction of butadiene with OH radicals (6.68×10^{-11} cm^3/mol per second),
- k_{ethene} is the rate constant for the reaction of ethene with OH radicals (8.5×10^{-12} cm^3/mol per second),
- M_{ethene} is the molecular weight of ethene (28.1 g/mol), and
- $M_{butadiene}$ is the molecular weight of butadiene (54 g/mol).

Because of its high reactivity, butadiene will be particularly important to photochemical ozone formation close to its sources of release. As it moves away from these sources, butadiene reacts with both hydroxyl radicals and ozone to form products such as formaldehyde, which are also active in the photochemical formation of ozone.

Various published reactivity values for butadiene and other selected VOCs are presented by Paraskevopoulos *et al.* (1995). The use of a maximum incremental reactivity (MIR) scale has been recommended by Carter (1994) as optimal when applied to the wide variety of conditions where ozone is sensitive to VOCs, being fairly robust to the choices of scenarios used to derive it.

Recently, butadiene was one of the VOCs identified in the Canadian 1996 NO_x/VOC Science Assessment as part of the Multi-Stakeholder NO_x/VOC Science Program (Dann and Summers, 1997). Based on air measurements taken at nine urban and suburban sites in Canada from June to August from 1989 to 1993, butadiene was ranked 60th of the most abundant non-methane hydrocarbon and carbonyl species. Based on these measurements and on an MIR value of approximately 10 mol ozone/mol carbon, butadiene represented approximately 0.9% of the total volatile organic carbon reactivity and was ranked 26th when sorted by the total volatile organic carbon reactivities. Total volatile organic carbon reactivity denotes the ability of organic compounds to contribute to the formation of ozone.

Therefore, because of its high reactivity and moderate concentrations encountered in Canada, butadiene plays a role in the photochemical formation of ground-level ozone.

3.0 Assessment of "Toxic" under CEPA 1999

3.1 CEPA 1999 64(a): Environment

The environmental risk assessment of a PSL substance is based on the procedures outlined in Environment Canada (1997a). Analysis of exposure pathways and subsequent identification of sensitive receptors are the basis for selection of environmental assessment endpoints (e.g., adverse reproductive effects on sensitive fish species in a community). For each endpoint, a conservative Estimated Exposure Value (EEV) is selected and an Estimated No-Effects Value (ENEV) is determined by dividing a Critical Toxicity Value (CTV) by an application factor. A hyperconservative or conservative quotient (EEV/ENEV) is calculated for each of the assessment endpoints in order to determine whether there is potential ecological risk in Canada. If these quotients are less than one, it can be concluded that the substance poses no significant risk to the environment, and the risk assessment is completed. If, however, the quotient is greater than one for a particular assessment endpoint, then the risk assessment for that endpoint proceeds to an analysis where more realistic assumptions are used and the probability and magnitude of effects are considered. This latter approach involves a more thorough consideration of sources of variability and uncertainty in the risk analysis.

3.1.1 Assessment and measurement endpoints

Butadiene enters the Canadian environment mainly from natural and anthropogenic combustion sources, notably vehicle emissions, and from industrial on-site releases. Almost all releases to the ambient environment are to air, with very small amounts released to water and soil.

Given its physical/chemical properties, butadiene undergoes various degradation processes in air. When released to water or soil, it can undergo various biological and physical degradation processes. In addition, much of the butadiene released onto soil can be expected to volatilize to air. Butadiene is not bioaccumulative or persistent in any compartment of the environment, although its continual release from automotive and other combustion sources can lead to chronic exposure of biota.

Based on the sources and fate of butadiene in the ambient environment, biota are expected to be exposed to butadiene primarily in air. Some exposure in water or in soil is possible. Butadiene does not bioaccumulate, and it should largely be found in the gas phase in air or dissolved phase in water. Therefore, the focus of the environmental risk characterization will be on terrestrial and aquatic organisms exposed directly to ambient butadiene in air and water and on soil organisms exposed to butadiene in soil.

3.1.1.1 Aquatic

Experimental data for toxicity of butadiene to aquatic organisms are not available (Section 2.4.1). Modelled data are available for algae, one species of crustacean (acute and chronic exposures) and six species of fish (acute and chronic exposures). Experimental data are available for the related substances 1,3-pentadiene (algae, crustacean, fish) and isoprene (fish).

Algae are primary producers in aquatic systems, forming the base of the aquatic food chain, while zooplankton, including crustaceans, are key consumers and are themselves consumed by many species of invertebrates and vertebrates. Fish are consumers in aquatic communities and are themselves eaten by piscivorous fish, birds and mammals.

Therefore, although limited, the available studies cover an array of organisms from different taxa and ecological niches and are considered adequate for an assessment of risks to aquatic

biota. The single most sensitive response for all of these endpoints is considered as the CTV for the risk characterization for aquatic effects.

3.1.1.2 Terrestrial

Data on terrestrial toxicity are available for six species of plants (experimental) and one soil invertebrate (modelled) (Section 2.4.1). Data pertinent to terrestrial vertebrate wildlife are available from several mammalian toxicology studies (Section 2.4.3).

Terrestrial plants are primary producers, provide food and cover for animals, and provide soil cover to reduce erosion and moisture loss. Invertebrates are an important component of the terrestrial ecosystem, consuming plant and animal matter while serving as forage for other animals. Vertebrate wildlife are key consumers in most terrestrial ecosystems.

Therefore, although quite limited, the available toxicity studies include organisms from different taxa and ecological niches and are considered adequate for an assessment of risks to terrestrial biota using hyperconservative exposure scenarios. The most sensitive responses for plants, invertebrates and vertebrates (acute and chronic exposure) will be used as CTVs for the risk characterization for terrestrial effects.

3.1.2 Environmental risk characterization

3.1.2.1 Aquatic effects

The lowest estimated concentration associated with aquatic toxicity for butadiene is 2.2 mg/L (2200 µg/L), based on a 16-day EC_{50} for *Daphnia* reproduction, derived through QSAR modelling; this value is used as the CTV. To calculate an ENEV, an application factor of 100 has been selected to account for extrapolation from laboratory to field conditions, inter- and intraspecies variability, extrapolation from an EC_{50} to a no-effect concentration and the fact that, although there are chronic and acute data for a variety of aquatic organisms and corroborating data for other substances, all the information has been obtained by QSAR modelling rather than experimentation. This yields an ENEV of:

$$\text{ENEV for water} = \frac{2200\ \mu g/L}{100}$$

$$= 22\ \mu g/L\ (0.022\ mg/L)$$

Since no empirical data are available for concentrations of butadiene in ambient waters in Canada, the assessment was based on predicted concentrations. For the industrial site having the highest reported air concentration, the equilibrium concentration in water was predicted to be 9.3×10^{-3} µg/L. This concentration can be used as the EEV in estimating the likelihood of risk in water.

A hyperconservative quotient can thus be calculated as follows:

$$\text{Quotient for water} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{9.3 \times 10^{-3}\ \mu g/L}{22\ \mu g/L}$$

$$= 4.2 \times 10^{-4}$$

Because the hyperconservative quotient is less than 1, this substance is unlikely to cause a harmful effect on populations of organisms in the ambient aquatic environment.

Extensive effluent monitoring data are available for the major producer and user of butadiene in Canada. Concentrations in raw, undiluted effluents were almost always below the detection limit (2839 samples analyzed with a detection limit of 1 µg/L, and 789 samples analyzed with a detection limit of 50 µg/L). Only two samples of undiluted effluents had measurable concentrations (80 and 130 µg/L) that exceeded the ENEV. It can therefore be expected that risks to aquatic organisms in receiving waters are low.

3.1.2.2 Terrestrial effects

3.1.2.2.1 Plants

The lowest concentrations of airborne butadiene associated with toxicity are a 21-day No-Observed-Effect Concentration (NOEC) of 22.1 mg/m^3 and a 21-day Lowest-Observed-Effect Concentration (LOEC) of 221 mg/m^3. These levels of exposure caused no effect on cotton, tomato and coleus plants and slight effects in cotton and tomato plants exposed for 21 days, respectively.

An ENEV for terrestrial plants can be derived from the LOEC of 221 mg/m^3. An application factor of 100 has been selected to account for extrapolation from laboratory to field conditions, inter- and intraspecies variability, extrapolation from a LOEC to a no-effect concentration and the fact that, although there are data for several plant species, there is only one laboratory study confirming these effects. This yields an ENEV of:

$$\text{ENEV for air} = \frac{221 \text{ mg/m}^3}{100}$$

$$= 2.21 \text{ mg/m}^3 \text{ (or } 2210 \text{ µg/m}^3\text{)}$$

A similar ENEV could be obtained using the no-effect level of 22.1 mg/m^3 and an application factor of 10 (no extrapolation to a no-effect concentration required).

At this time, the highest reported or predicted gas-phase concentration in outdoor air in Canada is 28 µg/m^3 (reported at one site near the major producer and user of butadiene).

A hyperconservative quotient can thus be calculated as follows:

$$\text{Quotient for air} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{28 \text{ µg/m}^3}{2210 \text{ µg/m}^3}$$

$$= 1.3 \times 10^{-2}$$

Because the hyperconservative quotient is less than 1, it is unlikely that butadiene is causing harm to populations of terrestrial plants through exposure in ambient air.

3.1.2.2.2 Soil invertebrates

The only soil toxicity concentration for butadiene is 335 mg/kg dry soil weight, which is a chronic 14-day LC_{50} earthworm survival value estimated using QSAR. An application factor of 1000 has been selected to account for extrapolation from laboratory to field conditions, inter- and intraspecies variability, extrapolation from an LC_{50} to a no-effect concentration and the fact that there is only one value available, derived by QSAR. This yields an ENEV of:

$$\text{ENEV for soil} = \frac{335 \text{ mg/kg}}{1000}$$

$$= 0.335 \text{ mg/kg}$$

The highest estimated soil concentration is 7.5×10^{-6} mg/kg (dry weight), yielding a hyperconservative quotient of:

$$\text{Quotient for soil} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{7.5 \times 10^{-6} \text{ mg/kg}}{0.335 \text{ mg/kg}}$$

$$= 2.2 \times 10^{-5}$$

TABLE 6 Summary of risk quotients for environmental assessment of butadiene

Assessment endpoint	EEV	CTV	Application factor	ENEV	Risk quotient EEV/ENEV
Aquatic organisms	9.3×10^{-3} µg/L	2.2 mg/L, QSAR 16-day EC_{50}, *Daphnia* reproduction	100	22 µg/L	4.2×10^{-4}
Terrestrial plants	28 µg/m³	221 mg/m³, 21-day LOEC, foliar lesions to cotton, tomato	100	2210 µg/m³	1.3×10^{-2}
Soil invertebrates	7.5×10^{-6} mg/kg	335 mg/kg, QSAR 14-day LC_{50}, *Eisenia fetida*	1000	0.335 mg/kg	2.2×10^{-5}
Wildlife, acute exposure	28 µg/m³	268 g/m³, 2-hour LC_{50}, mouse	10	26.8 g/m³	1.0×10^{-6}
Wildlife, acute exposure	28 µg/m³	221 mg/m³, 7-hour LOEL, biochemical changes in mouse	10	22.1 mg/m³	1.3×10^{-3}
Wildlife, chronic exposure	1.0 µg/m³	13.8 mg/m³, 2-year LOEL, mouse ovarian atrophy	10	1380 µg/m³	7.2×10^{-4}

Because the hyperconservative quotient is less than 1, this substance is unlikely to cause a harmful effect on populations of soil organisms in the terrestrial environment.

3.1.2.2.3 Mammalian wildlife

Since concentrations of butadiene are highest in urban areas and around industrial sites, city-dwelling terrestrial organisms are considered to have the greatest potential for exposure. Small mammals such as deer mice are likely to have the highest exposure due to their rapid respiration rate and high metabolism. Although no data have been identified for wild animals, effects data are available for surrogates such as laboratory mammals.

Acute exposure

The most sensitive single-exposure lethality study identified for laboratory mammalian studies was a 2-hour inhalation LC_{50} for mouse of 268 g/m³ (Shugaev, 1969) (256 g/m³ for exposure of unknown duration; Batinka, 1966). This will be used as the CTV for the acute exposure of terrestrial wildlife to butadiene in air. An application factor of 10 has been selected to

account for extrapolation from laboratory to field conditions, inter- and intraspecies variability and extrapolation from an LC_{50} to a no-effect concentration. This yields an ENEV of:

$$\text{ENEV (acute)} = \frac{268\ \text{g/m}^3}{10}$$

$$= 26.8\ \text{g/m}^3$$

A worst-case quotient is calculated by dividing the acute EEV of 28 µg/m³ (the highest airborne concentration of butadiene measured in Canada) by the ENEV. The resulting hyperconservative quotient (acute) is:

$$\text{Quotient for wildlife} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{28\ \mu\text{g/m}^3}{26.8 \times 10^6\ \mu\text{g/m}^3}$$

$$= 1.0 \times 10^{-6}$$

Biochemical changes were reported in mice after exposure for 7 hours at concentrations of 100 ppm (221 mg/m³) or more (Deutschmann and Laib, 1989). An application factor of 10 has been selected to account for extrapolation from laboratory to field conditions, inter- and intraspecies variability and extrapolation from a LOEL to a no-effect concentration. This yields an ENEV of:

$$\text{ENEV (acute)} = \frac{221\ \text{mg/m}^3}{10}$$

$$= 22.1\ \text{mg/m}^3$$

A worst-case quotient is calculated by dividing the acute EEV of 28 µg/m³ (the highest air concentration of butadiene measured in Canada) by the ENEV. The resulting hyperconservative quotient (acute) is:

$$\text{Quotient for wildlife} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{28\ \mu\text{g/m}^3}{22.1 \times 10^3\ \mu\text{g/m}^3}$$

$$= 1.3 \times 10^{-3}$$

Because the hyperconservative quotients are less than 1, it is unlikely that butadiene emissions will cause acute adverse effects on populations of terrestrial wildlife in Canada.

Chronic exposure

The most sensitive study identified for laboratory mammals was a chronic study in which mice were exposed to butadiene for 2 years (6 hours per day for 5 days per week). NTP (1993) reported that the LOEL was 6.25 ppm (13.8 mg/m³). Butadiene induced adverse toxic effects, including ovarian atrophy, in mice at this concentration. Mice were much more sensitive than other mammals to exposure to butadiene.

The ENEV is derived by dividing the CTV by an application factor of 10 to account for extrapolation from laboratory to field conditions, inter- and intraspecies variability and extrapolation from a LOEL to a no-effect concentration. This yields an ENEV of:

$$\text{ENEV (chronic)} = \frac{13.8\ \text{mg/m}^3}{10}$$

$$= 1380\ \mu\text{g/m}^3$$

A worst-case quotient is calculated by dividing the chronic EEV of 1.0 µg/m³ (the 95th percentile for extensive air monitoring data under the NAPS program) by the ENEV. The resulting hyperconservative quotient (chronic) is:

$$\text{Quotient for wildlife} = \frac{\text{EEV}}{\text{ENEV}}$$

$$= \frac{1.0\ \mu\text{g/m}^3}{1380\ \mu\text{g/m}^3}$$

$$= 7.2 \times 10^{-4}$$

Because the hyperconservative quotient is less than 1, it is unlikely that butadiene emissions will cause chronic adverse effects on populations of terrestrial wildlife in Canada.

A summary of the values used in the environmental risk characterization of butadiene is presented in Table 6.

3.1.2.3 Discussion of uncertainty

There are a number of potential sources of uncertainty in this environmental risk assessment. Regarding effects of butadiene on terrestrial and aquatic organisms, there is uncertainty concerning the extrapolation from available toxicity data to potential ecosystem effects. To account for this uncertainty, application factors were used in the environmental risk analysis to derive ENEVs.

All data for effects on aquatic and soil organisms are derived through QSAR modelling, and there is only one experimental study for terrestrial plants. Nonetheless, corroborating data are available for structurally and functionally similar substances, lending support to the modelled data.

Regarding environmental exposure, there could be concentrations of butadiene in Canada that are higher than those identified and used in this assessment. However, the air measurements used in this assessment are considered acceptable because they were selected from an extensive set of recent air monitoring data of urban and other sites, including key industrial manufacturing sites. Thus, available data on atmospheric concentrations are considered representative of the highest concentrations likely to be encountered in air in Canada.

Concentrations in water are expected to be low because of the limited releases to this medium and the limited partitioning of butadiene from air into water. Since no measurements of butadiene are available in ambient water, concentrations were predicted by modelling. Extensive monitoring data are available for the key industrial plant producing and using butadiene and indicate that butadiene is unlikely to occur in concentrations of concern even in undiluted effluents.

Despite some data gaps regarding the environmental effects of and exposure to butadiene, the data available at this time are considered adequate for making a conclusion on the environmental risk of butadiene in Canada.

3.2 CEPA 1999 64(b): Environment upon which life depends

Butadiene is generally released to air, and its properties largely preclude its partitioning into other compartments. There is thus a potential for butadiene to be involved in critical atmospheric processes. Butadiene does not deplete stratospheric ozone, and its potential contribution to climate change is negligible. Butadiene is more reactive (POCP of 407) than compounds such as ethene that are recognized as important in the formation of ground-level ozone. Given its high reactivity and the concentrations measured in air in Canada, butadiene represented approximately 0.9% of the total volatile organic carbon reactivity, ranking it 26th among non-methane hydrocarbons and carbonyl compounds contributing to the formation of ground-level ozone (Dann and Summers, 1997). Butadiene may therefore be important in the photochemical formation of ground-level ozone in urban areas.

3.3 CEPA 1999 64(c): Human health

3.3.1 Estimated population exposure

The principal source of environmental exposure to butadiene is air. Although few data were identified regarding levels in drinking water and food, intake of butadiene in these media is expected to be negligible in comparison with that in air because of its physical/chemical properties (e.g., vapour pressure and partition coefficients) and environmental release patterns (i.e., principally atmospheric emissions).

Twenty-four-hour average concentrations of butadiene were measured in 9168 samples of outdoor air between 1989 and 1996 under the NAPS program (Dann, 1997). Sampling sites were located in rural, suburban and urban areas. In the absence of potential indoor sources, and if it is assumed that the general population in Canada is exposed to similar concentrations of butadiene, 50% of the population can be expected to be exposed to 24-hour average concentrations of up to 0.21 $\mu g/m^3$, while 95% of the population can be expected to be exposed to 24-hour average concentrations of up to 1.0 $\mu g/m^3$.

The general population in urban areas is exposed to higher concentrations of butadiene on an ongoing basis. The 90th and 95th percentile values of the distribution of concentrations in 2913 samples from nine urban NAPS sites were 0.8 $\mu g/m^3$ and 1.1 $\mu g/m^3$, respectively. Butadiene was detected in 98% of 1576 samples from four reasonable worst-case urban NAPS sites, at a mean concentration of 0.5 $\mu g/m^3$. In the absence of potential indoor sources, and if it is assumed that highly exposed subgroups within the population in Canada are exposed to concentrations of butadiene similar to those at the reasonable worst-case sites, 50% of the population can be expected to be exposed to 24-hour average concentrations of up to 0.40 $\mu g/m^3$, while 95% of the population can be expected to be exposed to 24-hour average concentrations of up to 1.3 $\mu g/m^3$.

Exposures from ambient air may be substantially higher for populations in the vicinity of point sources. Concentrations of butadiene were measured at distances between 1 and 3 km downwind of an industrial point source of discharge to the atmosphere in Sarnia, Ontario (MOEE, 1995). If these concentrations can be considered as a worst case of the ongoing exposure of nearby residents, and in the absence of additional potential indoor sources, 50% of the population in the vicinity of this source can be exposed to short-term concentrations of up to 0.62 $\mu g/m^3$, and 95% of this population can be exposed to short-term concentrations of up to 6.4 $\mu g/m^3$.

Individuals may also be exposed to butadiene for short durations while at self-service gasoline filling stations or in parking garages. Estimates of average daily intake of butadiene by inhalation for various exposure scenarios indicate that intake is negligible while at self-service stations due to the infrequent occurrence and short duration of these exposures. Higher daily intakes are possible for commuters using personal motor vehicles and parking garages on a regular basis. However, these intakes are still much less than average daily intakes for the general population from inhalation of background concentrations of butadiene in outdoor and indoor air.

Although available Canadian data indicate that butadiene is detected with greater frequency in indoor air than in outdoor air, there are insufficient data to characterize the distributions of concentrations of butadiene in various indoor environments. In general, butadiene is detected more frequently and at higher concentrations in indoor environments contaminated by ETS than in areas where smoking does not occur. In non-smoking indoor areas in Canada, the distributions of concentrations of butadiene are likely to be similar to the distributions of concentrations in the outdoor air samples from the NAPS program. Non-smokers who spend a considerable proportion of their time in indoor environments where ETS is present can be exposed to concentrations of butadiene that are an order of magnitude higher than the average levels in the outdoor air. Moderate tobacco use (e.g., 20 cigarettes per day) can increase the daily intake of butadiene by smokers by five times over the daily intake by non-smokers in ETS-contaminated indoor locations. The daily intake of butadiene by smokers can be 100 times greater than the daily intake of non-smokers who are not exposed to ETS.

3.3.2 Hazard characterization

As discussed in Section 2.4.2, although metabolism of butadiene appears to be qualitatively similar across species, there are extensive data that indicate that the putatively active epoxide metabolites are formed to a greater degree in mice than in rats. Similarly, although *in vivo* data are limited, humans appear to metabolize butadiene to the mono- and diepoxide metabolites to a much lesser extent than mice. However, based on the observed variability in the formation of adducts of hemoglobin with butadiene metabolites in occupationally exposed human populations, there appears to be interindividual variation in humans, which is likely related to polymorphism for genes that code for enzymes involved in the metabolism of butadiene. The weight of evidence for the carcinogenicity, genotoxicity and non-neoplastic effects of butadiene needs to be considered, therefore, in the context of these interspecies and interindividual variations.

3.3.2.1 Weight of evidence for carcinogenicity and genotoxicity

Data supporting the interspecies differences in production of active epoxide metabolites are in concordance with the observed difference in sensitivity between mice and rats (at least for the few strains investigated) to butadiene-induced carcinogenicity, in that the substance appears to be much more potent in mice than in rats. Although butadiene was a multi-site carcinogen in both mice and rats at all exposure levels tested (Hazleton Laboratories Europe Ltd., 1981a; NTP, 1984, 1993; Irons *et al.*, 1989), the concentrations that induced tumours in the only study available in rats were much greater than those that were tumorigenic in mice (i.e., ≥1000 ppm versus ≥6.25 ppm).

Species differences in sensitivity to genetic effects induced by butadiene have also been observed. Although mutagenic in somatic cells of both mice and rats, the mutagenic potency of butadiene was greater in mice. Other genotoxic endpoints (chromosomal aberrations, sister chromatid exchanges and micronuclei) were noted in somatic cells of mice but not in those of rats exposed to much higher concentrations. Butadiene was genotoxic in germ cells of male mice in multiple assays, while negative results were obtained in the single dominant lethal study in rats. Unlike the observations with the parent compound, however, there is little evidence that there are species differences in the sensitivity to genotoxic effects induced by the epoxide metabolites of butadiene (EB, DEB and EBdiol), although there was some indication of interstrain variability. These data suggest that interspecies differences in sensitivity to butadiene-induced genotoxicity are related to quantitative differences in the formation of active metabolites.

There is also limited evidence of the genotoxicity of butadiene in exposed workers; although data are not completely consistent, increased frequencies of chromosomal aberrations, sister chromatid exchanges, and *hprt* mutations and decreased DNA repair capability have been reported in some studies of workers in the monomer and/or styrene-butadiene rubber manufacturing industries (Legator *et al.*, 1993; J.B. Ward *et al.*, 1994, 1996; Au *et al.*, 1995; Tates *et al.*, 1996; Hallberg *et al.*, 1997; Ward, 1997a, 1997b; Srám *et al.*, 1998). The discrepancy in the results may be due to the use of different methods for the detection of mutations or differences in exposure levels. In addition, since sensitivity to induction of genetic effects by butadiene and its metabolites has been linked to genotype for glutathione-S-transferase enzymes in several *in vitro* and a few *in vivo* studies, interpretation of the inconsistent observations in the available database is complicated by the lack of information on genotype for most of the small populations examined.

There have been several epidemiological investigations of the carcinogenicity of butadiene that serve as a basis for assessment of the weight

of evidence for causality based on traditional criteria. In the most recent cohort study (Delzell *et al.*, 1995), which is also the largest and most comprehensive investigation conducted to date and that in which exposure was most extensively characterized, an association between exposure to butadiene in the styrene-butadiene rubber industry and leukemia was observed (i.e., there was a quantifiable exposure–response relationship). SMRs for leukemia were elevated for the overall cohort of workers from eight plants; the strength of this association was generally greater when specific subgroups with greater potential for exposure were considered. In addition, there was an increase in the RR for leukemia with increased cumulative exposure to butadiene in workers from the six plants for which exposure was best characterized. The association between leukemia and exposure to butadiene remained when the potential role of two other substances present in the work environment (i.e., styrene and benzene) was considered. Although further refinement of the estimates of exposure at one of these plants resulted in increases for several job categories (Macaluso *et al.*, 1997), it is unlikely that these changes would affect the relative ranking of the categories and analyses in which exposed workers were compared with "non-exposed" workers (Gerin and Siemiatycki, 1998); therefore, these results are not inconsistent with the association observed by Delzell *et al.* (1995).

However, no increase in mortality due to leukemia was observed in studies of workers involved in the production of butadiene monomer who were not concomitantly exposed to the other substances present in the styrene-butadiene rubber industry (E.M. Ward *et al.*, 1995, 1996; Divine and Hartman, 1996). Although there was some evidence of increased mortality due to lymphosarcoma and reticulosarcoma in the subgroup of workers potentially exposed to the highest concentrations of butadiene in the largest of these investigations, there was no association with duration of employment or estimated cumulative exposure (based on qualitative ranking of potential for exposure). Although mortality due to lymphosarcoma was non-significantly elevated in some process groups in the styrene-butadiene rubber cohort (Delzell *et al.*, 1995), there were no consistent patterns (other than for leukemia), even when currently accepted terminology for lymphohematopoietic cancers was used (Sathiakumar *et al.*, 1998).

The traditional criterion of consistency for the observed association between exposure to butadiene and leukemia is fulfilled, at least in part, in that similar excesses were observed among plants in the large cohort study of styrene-butadiene rubber workers (Delzell *et al.*, 1995); i.e., there is internal consistency. A similar exposure–response was also noted in an independant nested case–control study of mostly the same population in which different exposure assessment methodology was employed (Matanoski *et al.*, 1997). Observation of external consistency with results of other cohort studies of styrene-butadiene rubber workers is largely precluded, in view of the scope of the large epidemiological cohort study that included a large proportion of all of the styrene-butadiene rubber workers in North America. Indeed, it is difficult to envisage additional studies in this occupational group that would contribute meaningfully to weight of evidence for consistency of the observed association.

One criterion for causality of observed associations in epidemiological studies, namely coherence, may not have been adequately fulfilled, in view of the difference in the specific form of lymphohematopoietic cancer in excess in available investigations for the two principal types of populations of workers studied. Indeed, increases in lymphosarcoma and reticulosarcoma have been observed in monomer production workers, whereas increases in leukemia have been observed in styrene-butadiene rubber workers. Although it is plausible that this difference may be related to variation in the extent of information available for characterization of exposure or to the nature of exposures in the two industries, this has not been systematically investigated. There is also the possibility of misclassification of cause of death on death certificates (although Sathiakumar

et al. [1998] did not observe an association with forms of lymphohematopoietic cancer other than leukemia in the large cohort of styrene-butadiene rubber workers when causes of death were examined using current terminology). The potential for transformation of one form of lymphohematopoietic cancer to another (e.g., non-Hodgkin's lymphoma to leukemia) has also been noted (Sathiakumar *et al.*, 1998). In addition, available data for the large study of styrene-butadiene rubber workers were insufficient to determine if butadiene was causally associated with a specific form of leukemia. Moreover, it is noteworthy that these different tumours observed in styrene-butadiene rubber workers and monomer production workers are of the same organ system, and perhaps even share the same pluripotential stem cell.

An association between exposure to butadiene and the induction of leukemia is also biologically plausible. The hematopoietic system is a target for butadiene-induced effects in rodents (i.e., lymphocytic lymphomas [NTP, 1993], cytogenetic effects in bone marrow [Cunningham *et al.*, 1986; Irons *et al.*, 1986a, 1987; Tice *et al.*, 1987; NTP, 1993; Leavens *et al.*, 1997] and suppression of stem cell differentiation [Irons *et al.*, 1996]). Aneuploidy, which is believed to be associated with leukemia in humans, has been induced in human lymphocytes exposed *in vitro* to the mono- and diepoxide metabolites of butadiene (Vlachodimitropoulos *et al.*, 1997; Xi *et al.*, 1997). Moreover, the presence of relevant metabolizing enzymes in progenitor cells believed to be important targets for the induction of leukemia in humans (i.e., $CD34^+$ cells) has been demonstrated in studies of the metabolism of benzene (a documented human leukemogen) (Schattenberg *et al.*, 1994; Ross *et al.*, 1996b) (although exposure of human $CD34^+$ cells to EB at "physiologically relevant concentrations" did not alter cytokine-induced clonogenic response, an early change frequently observed in the development of leukemia; Irons *et al.*, 1996; Irons, 1998). Therefore, available data also support the biological plausibility of an association between exposure to butadiene and leukemia observed in humans, although the active metabolite has not been identified.

Therefore, although not completely convincing in their own right, the available epidemiological studies of the association between leukemia and exposure to butadiene in occupationally exposed human populations fulfil several of the traditional criteria for causality, including strength of association (RR of 4.2 in the highest exposure group [based on five cases], which would be considered moderately strong), quantifiable exposure–response relationship, temporal relationship (the critical investigation [i.e., Delzell *et al.*, 1995] is a historical cohort study), biological plausibility and, to some degree, consistency, although the criterion for coherence is not fully satisfied.

Assessment of the weight of evidence for carcinogenicity in human populations should not, however, be considered in isolation from the extensive supporting data on carcinogenicity, genotoxicity and inter- and intraspecies variations in metabolism and response. The association between exposure to butadiene and development of cancer is supported by limited evidence of genetic damage in exposed workers, as well as the wealth of evidence that butadiene is carcinogenic and/or genotoxic in all species of experimental animals tested (mice, rats and hamsters), inducing a wide range of tumours and genetic damage at relatively low concentrations in mice (i.e., within the same order of magnitude as current occupational health limits). Moreover, while there are quantitative differences in the potency of the substance to induce tumours in various species, likely related to observed quantitative differences in metabolism, there are indications of considerable interindividual variations in the metabolism of butadiene in the human population, consistent with expectations for a complex metabolic pathway.

Based on the evidence of an association between exposure in the occupational environment and leukemia that fulfils several of the traditional criteria for causality of associations

observed in epidemiological studies, supporting limited data on genotoxicity in human populations and the overwhelming weight of evidence of carcinogenicity and genotoxicity at relatively low concentrations in some species of experimental animals, butadiene is considered highly likely to be carcinogenic in humans.

Although relevant data in humans are limited, the results of *in vivo* studies in experimental animals indicate that butadiene induces mutations in somatic cells and male germ cells as well as male-mediated heritable clastogenic damage. While most of the studies have been conducted in mice, rats appear to be less sensitive to these effects, which is consistent with species differences in metabolism. However, in view of the likely considerable heterogeneity in the metabolism of butadiene in human populations, butadiene is considered a likely human somatic and germ cell genotoxicant.

3.3.2.2 Weight of evidence for non-neoplastic effects

The available data on effects of butadiene other than carcinogenicity or genotoxicity are limited. Based on the limited data available, species differences in the ability of butadiene to induce other non-neoplastic effects again appear to be consistent with variations in metabolism of butadiene to active metabolites. However, butadiene is of low acute toxicity in both rats and mice, in contrast to its ability to induce cancer and genetic damage at relatively low concentrations in mice.

Hematological effects suggestive of macrocytic anemia have been consistently observed in mice (two strains) following short-term, subchronic or chronic exposure to butadiene at concentrations similar to or lower than those that induced general toxicity (as indicated by decreased body weight gain and increased organ weights) (Irons *et al.*, 1986a, 1986b; NTP, 1993; Bevan *et al.*, 1996). For example, changes in hematological parameters were noted in mice exposed to ≥62.5 ppm (≥138 mg/m^3) butadiene for 9 months or longer in the NTP bioassay. Butadiene also induced effects on bone marrow (including atrophy, decreased cellularity, regeneration and alterations in stem cell development) in mice (Irons *et al.*, 1986a, 1986b; Leiderman *et al.*, 1986; NTP, 1993), although available data are inadequate to assess the potential effects on immune system function. While effects on the blood and bone marrow have not been reported in rats in recent investigations (including the only identified chronic bioassay; Hazleton Laboratories Europe Ltd., 1981a), the database is considerably more limited. In addition, the lack of observation of hematotoxicity in rats may again reflect the species differences in metabolism. Although the available epidemiological studies are too limited to assess the hematotoxicity in humans, available data support the hematopoietic system being a critical target for butadiene-induced toxicity, since the lymphohematopoietic system is a target for butadiene-induced leukemia in humans. However, it has not been established if the non-neoplastic effects observed in animals may be preliminary to, or associated with, the development of lymphohematopoietic cancers.

The reproductive organs are also critical targets of butadiene-induced non-neoplastic effects in mice. Ovarian atrophy, the severity and incidence of which increased with concentration or duration of exposure, was observed at all concentrations (i.e., ≥6.25 ppm [≥13.8 mg/m^3]) in the chronic bioassay conducted by the NTP (1993); in all exposure groups, the level of degeneration at 2 years, characterized by lack of oocytes, follicles or corpora lutea, was incompatible with reproductive capacity. Although recent re-examination of some of the tissue samples indicated that the atrophy observed in the ovaries may be related to senile changes (Davis, 1998), it may be that butadiene is exacerbating these changes. It should be noted, though, that the incidence of these lesions was increased as early as 9 months (although the slides from these interim sacrifices have not been re-examined). That butadiene is causally associated with these lesions is also difficult to dismiss on the basis

of currently available data, in view of the consistency with the results of other studies, including the earlier NTP (1984) bioassay and a subchronic study at higher concentrations (Bevan *et al.*, 1996) in which such lesions were also observed, the presence of a clear dose–response relationship and biological plausibility. Based on the observation of depletion of ovarian follicles and alkylation with ovarian macromolecules in mice following intraperitoneal administration of the monoepoxide or diepoxide metabolite and in rats administered the diepoxide (Doerr *et al.*, 1995), it is possible that the ovarian toxicity is mediated through generation of the active epoxide metabolites.

Testicular atrophy was noted only in male mice exposed to concentrations greater than those that induced effects in females (NTP, 1993). Consistent with metabolic differences, butadiene did not induce ovarian or testicular toxicity in the limited number of available studies in rats, although, as noted above, the diepoxide metabolite was ovotoxic in both species (Doerr *et al.*, 1995, 1996). Although available data are limited, there is no conclusive evidence that butadiene is teratogenic in mice or rats following maternal or paternal exposure or that it induces significant fetal toxicity at concentrations below those that are maternally toxic. Available epidemiological data are inadequate for evaluation of potential reproductive or developmental toxicity; in fact, none of the identified analytical studies was conducted in women. However, in view of the qualitative similarities in the metabolism of butadiene in mice, rats and humans and the likely variation across the general population associated with genetic polymorphism for the relevant enzymes, and on the basis of the observed ovarian toxicity in butadiene-exposed mice, butadiene is considered to be a possible reproductive toxicant in humans, although additional work to clarify the relevance of these observed effects is clearly desirable.

Available data on other systemic or organ-specific effects are inadequate to determine if such effects might be considered critical.

3.3.3 Exposure–response analyses

Since air is the principal route of exposure to butadiene in the general environment (available data indicate that other routes contribute negligibly), quantitation of exposure–response for cancer and non-cancer effects is limited to exposure by inhalation.

In order to eliminate the uncertainty associated with extrapolation from animal species, quantitative measures of carcinogenic potency (i.e., tumorigenic concentrations, or TCs)[4] have been developed on the basis of available epidemiological data. This is based on the conclusion that the weight of evidence for an association between butadiene and leukemia satisfies several of the traditional criteria for causality in epidemiological studies. However, uncertainties in the exposure estimates for the critical cohort of workers as well as confounding or effect-modifying aspects that could impact on quantitative estimates of risk are recognized. In view of these factors and to serve as a basis for comparison, quantitative measures of cancer potency have also been developed on the basis of results of long-term bioassays in rats and mice, with those in mice being considered justifiably conservative, considering the likely heterogeneity in metabolic transformation of butadiene in humans. (See discussion of relevance of specific

[4] The potency estimate for carcinogenicity adopted in the Priority Substances Program is determined by calculating the dose or concentration associated with an increase in cancer incidence or mortality of an appropriate percentage. When based on toxicological data from studies in experimental animals, a 5% increase is generally chosen, as these values usually lie within or close to the observable range (i.e., a TC_{05} is calculated). When epidemiological data form the basis for derivation of a tumorigenic concentration, the percent increase selected is that which falls within the area of the exposure–response curve that represents the majority of the observable data; this is often less than 5%. In the case of butadiene, the carcinogenic potency calculated on the basis of modelling of epidemiological data (as described herein) was considered to be best defined as a 1% increase in mortality due to leukemia (i.e., a TC_{01}).

tumour types in animals to humans in Section 3.3.3.1.2.)

In addition to inducing tumours at multiple sites in experimental animals, there is also sufficient evidence that butadiene is genotoxic in somatic and germ cells and induces reproductive and hematological effects in animals. As a measure of exposure–response for non-cancer effects, where considered appropriate, benchmark concentrations[5] have been calculated on the basis of data from long-term studies in mice.

Several physiologically based pharmacokinetic (PBPK) models have been developed as a basis for reducing uncertainty in interspecies extrapolations for butadiene by various groups of investigators. However, none of the models currently available has adequately accounted for the distribution of metabolites in the compartments included; the principal researchers in this field have concluded that there are likely more factors involved in butadiene metabolism than have been included in the models developed to date (Csanády *et al.*, 1996; Sweeney *et al.*, 1997). In addition, none of the models has included the formation of EBdiol, a putatively active metabolite that is believed to be important in humans, since it has been observed to bind to hemoglobin to a greater degree than EB in workers exposed to butadiene. Nor has bone marrow been incorporated as a compartment, although it appears to be a target site of butadiene-induced toxic effects. Moreover, none of the PBPK models has been validated in humans. For these reasons, therefore, such models have not been used to quantitatively account for interspecies variations in metabolism in the quantitation of exposure–response for critical endpoints based on studies in experimental animals presented here. In addition, owing to its relatively slow metabolism, butadiene achieves a steady state during prolonged inhalation exposure. On this basis, exposures of the same concentration and duration would be expected to result in equivalent toxicity across species, and no interspecies scaling to account for variations in inhalation rate to body weight ratios or body surface areas between humans and animals have been incorporated.

3.3.3.1 Carcinogenicity

3.3.3.1.1 Estimated potency based on epidemiological data

In only one epidemiological investigation of the association between butadiene and leukemia have data on exposure of the study population been sufficiently characterized to permit quantitation of exposure–response (Delzell *et al.*, 1995). The Delzell *et al.* (1995) study also presents results for the largest cohort studied to date (including subjects from eight plants, six of which were included in the exposure–response analyses); it is also considered to subsume the observations of mortality in workers at these plants reported previously by other researchers (i.e., Meinhardt *et al.*, 1982; Matanoski *et al.*, 1990, 1993; Santos-Burgoa *et al.*, 1992), because of the considerable overlap in the cohort definition. The exposure assessment of study subjects was of extremely high quality, being very thorough and based on industrial hygiene monitoring data (although limited and used primarily for comparison with estimated concentrations), research of plant records concerning work histories, processes and local emissions, and consultation with staff from each plant, and is, therefore, considered appropriate for quantification of exposure–response. For comparison with estimates based on the data from the cohort study, carcinogenic potency was also calculated on the basis of the results of the case–control study nested within essentially the same population of workers (Matanoski *et al.*, 1997), although data available in the published report were too limited to permit detailed analysis here.

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[5] Similar to tumorigenic concentrations (TC_{05}s), benchmark concentrations for non-cancer effects (or BMC_{05}s), when based on data in experimental animals, represent the dose or concentration associated with a 5% increase in the incidence of an effect compared with controls.

Methods

The raw study data[a] for the six plants investigated by Delzell *et al.* (1995) were used to calculate the potency estimates. The data consisted of the cumulative occupational exposures to butadiene and styrene at each year of each subject's life (person-year), beginning with his entry into the cohort and terminating with death or other exit from the cohort. The data also contained information on race, age, calendar year and years since hire of each subject.

The response of interest was cases of death due to all forms of leukemia, as information on the specific type of leukemia was insufficient; only cases in which leukemia was considered the underlying cause of death were considered in these analyses. Exposure estimates were cumulative occupational exposures in ppm-years assumed to be incurred for 8 hours per day, 240 days per year over a 45-year working life.

The objective of this exposure–response analysis was to compute the carcinogenic potency, expressed as the TC_{01}, or the concentration of butadiene associated with a 1% excess probability of dying from leukemia. This analysis involved two stages. First, the relationship between exposure and the death rate due to leukemia within the cohort was modelled. This was accomplished by collapsing (or stratifying) the data into discrete exposure categories and then modelling the mean exposure in each category versus the death rates due to leukemia. In the second stage of analysis, the TC_{01} was calculated based on this exposure–response relationship and the background mortality rates in the Canadian population.

Exposure–response modelling:

In addition to stratifying by exposure, the data were stratified by race, age, calendar year, years since hire and styrene exposure in order to incorporate this information into the exposure–response relationship. Each of these variables was collapsed into a small number of discrete categories in order to reduce the number of strata, thereby improving model stability. These variables and their categories are presented in Table 7. Exposure, defined as the mean cumulative exposure per person-year, was calculated for person-years falling into each possible combination of the stratification variables.

The data were imported to Epicure (1993)[b] for exposure–response modelling. All fitted models were of the form:

$$RR = \frac{O}{E} = g\,(D(t))$$

where RR is the rate ratio, O and E are the observed and expected numbers of leukemia deaths, $D(t)$ is cumulative butadiene exposure up to time t, and g is the exposure–response model, which is constrained to pass through one at zero exposure. Four different models, discussed in more detail below, were fitted to the data. At the model-fitting stage, the expected number of deaths is calculated on the basis of the non-exposed person-years in the cohort, and not from Canadian population background rates.

Lifetime probability of death due to leukemia:

Once the fitted exposure–response model was obtained, the lifetime probability of death due

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[a] The cooperation of the sponsors and researchers for the Delzell *et al.* (1995) study in the provision of these data is gratefully acknowledged.

[b] Epicure is a collection of interactive programs used to fit models to epidemiological data. The specific program used to model the data for this cohort of styrene-butadiene rubber workers is called AMFIT, which is specially designed to model hazard functions for censored cohort survival data. The strength of Epicure lies in its ability to easily allow the background rate to depend on user-specified strata, such as age, calendar period and race.

TABLE 7 Stratification variables for exposure–response modelling of epidemiological data from Delzell *et al.* (1995)

Variable	Categories
Cumulative butadiene exposure (ppm-years)	0, >0–4, 5–9, 10–19, 20–29, 30–49, 50–99, 100–199, 200+
Cumulative styrene exposure (ppm-years)	0, >0–3, 4–6, 7–9, 10–19, 20–39, 40–59, 60–79, 80+
Race	black, white, other
Age	40–44, 45–49, ..., 75–79, 80+
Calendar period	1940–44, 1945–49, ..., 1990–95
Years since hire	0–4, 5–9, ..., 50–55

to leukemia was computed using lifetable methods taking into account the death rates in the Canadian population. The derivation of the formula used for the lifetime probability of death due to leukemia proceeds as follows.

Let $d(t)$ represent the exposure concentration of butadiene in ppm at age t years, and let $D(t)$ denote the cumulative exposure in ppm-years with:

$$D(t) = \int_o^t d(x)dx$$

This formulation of cumulative exposure allows for the possibility of non-constant exposure scenarios.

At a cumulative exposure of $D(t)$ ppm-years, the probability of dying from leukemia by age t is given by:

$$P(D(t);t) = 1 - \exp\left[\int_o^t h_R(D(x);x)S(x)dx,\right] \quad (1)$$

where $h_R(D(t);t)$ is the mortality rate from leukemia at age t given a cumulative exposure to butadiene of $D(t)$, and $S(t)$ is the probability of survival up to age t. Equation (1) follows from the argument that the probability of death by age t is equal to the probability of death at age t multiplied by the probability of surviving up until age t. In lifetable analysis, the mortality and survival rates are constant for each year, so the integral in (1) can be replaced by a summation over year.

Exposure to butadiene is assumed to augment the background rate of leukemia for the Canadian population in a multiplicative fashion. In other words, the mortality rate, given exposure to butadiene, is equal to the background exposure rate multiplied by the excess risk due to exposure to butadiene. This is known as the "proportional hazard" model and is expressed as:

$$h_R(D(t);t) = h(t)\cdot[g(D(t))] \quad (2)$$

where $h(t)$ is the background mortality rate from leukemia in the Canadian population, calculated from Canadian age-specific death rates[a] due to leukemia, and $g(D(t))$ is the fitted exposure–response model, or excess risk at age t.

The survival rate, $S(t)$, appearing in equation (1) is computed from Canadian age-specific death rates due to all causes, where the reported Canadian leukemia mortality rate is replaced by the modelled rate in order to incorporate exposure to butadiene. The formula describing the probability of survival up to age i is given by:

$$S_i = \exp\left[-\sum_{j=1}^{i} h_j^* - h_j + h_j g_j\right] \qquad (3)$$

where h_j^* and h_j are the Canadian mortality rates due to all causes and due to leukemia at age j, respectively, and $g_j = g(D(j))$ is the excess risk at age j.

Substituting equation (2) into (1), the lifetime probability of death due to leukemia is given by:

$$P(D(70); 70) = \sum_{i=1}^{70} h_i g_i S_{i-1}$$

where 1–70 years is the standard lifetime for a human.

Cancer potency (TC_{01}):

The TC_{01} is computed by determining the exposure $D(t)$ at which the excess risk is equal to 0.01. That is,

$$\frac{P(D(t);t) - P(0;t)}{1 - P(0;t)} = 0.01$$

If a constant exposure d is assumed for an individual from birth to age 70 years, then $d(t) = d$ ppm and the cumulative exposure $D(t) = d \cdot t$ ppm-years. The TC_{01} is then the ambient exposure level d (in ppm) at which the excess risk equals 0.01 at $t = 70$ years.

Lagged exposure analysis:

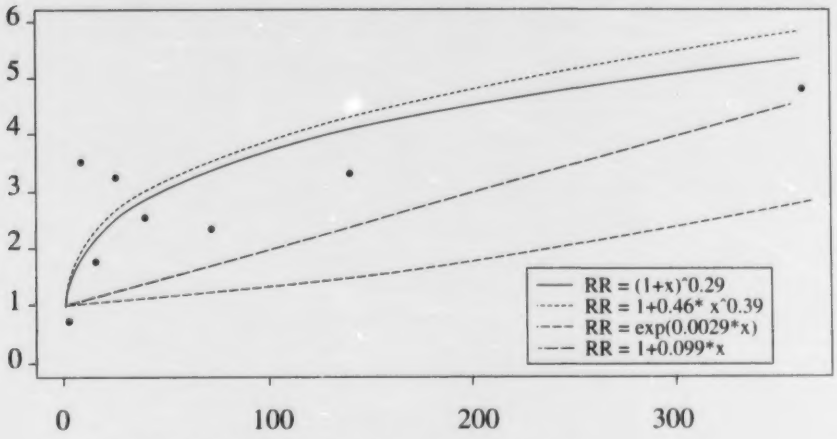
In separate analyses, exposures were lagged by $n = 2, 5, 10, 15, 20$ and 25 years to determine if the models would provide better fits if the most recent n years of exposure were ignored. An n-year lag was achieved by resetting an individual's cumulative exposure at each year to be equal to the exposure he had accumulated n years prior. In so doing, the last n years of exposure do not affect the probability of developing leukemia. The data were first stratified on unlagged cumulative exposure, and then the individual exposures were lagged. Thus, the number of strata remains constant when using different lag periods, and models with different lags may be directly compared (Preston *et al.*, 1987).

Validation study:

To assess the predictive power of the exposure–response models, a validation study was performed in which individuals in the cohort were divided randomly into two groups. The models were fit separately to both groups, and then a likelihood ratio test was performed to determine if the estimated parameters were equal. The process of dividing and fitting was repeated 1000 times to characterize the variability due to the random splitting process. If the models provided consistent fits, then the likelihood ratio test would be expected to reject at a rate equal to the desired significance level of the test (i.e., at a significance level of 0.05, the fitted parameters should be

[a] Mortality data were provided to Health Canada by Statistics Canada. The cooperation of the registrars of vital statistics in the provinces and territories of Canada who make mortality data available to Statistics Canada under federal–provincial agreements is gratefully acknowledged.

FIGURE 2 Observed rate ratios and fitted curves for leukemia in Delzell *et al.* (1995) study



Mean cumulative butadiene exposure per person-year (ppm-years)
Adjusted for age, calendar period, race, years since hire and styrene exposure.

significantly different 1 in 20 times). If the tests are significant more often than this, the confidence in the predictive power of the models is reduced.

Results

Exposure–response modelling:

Four different exposure–response models were examined and are presented in Table 8. These models are identical to those fitted in the Delzell *et al.* (1995) report except that model 2 is more general and flexible than the square root model used by those authors. Preliminary analysis indicated that all stratification variables except race significantly affected the model fit. Since race was only marginally insignificant, all variables were used to stratify the data prior to model fitting.

The four models were fitted while stratifying on race, age, calendar year, years since hire and styrene exposure. The results of the model fitting are displayed in Table 8. (N.B.: A smaller deviance roughly indicates a better fit.) A graphic representation of the data and the fitted models is shown in Figure 2. Judging from the model deviances and the shape of the curves relative to the data, especially in the low-dose region, model 1 provides the best fit to the data.

For purposes of comparison, the same models were fitted using the median exposure as per the Delzell *et al.* (1995) report. These analyses indicated that there is little difference between using mean or median exposures. Models including age as a multiplying factor of $e^{\gamma age}$ instead of as a stratification variable were also fitted, but these models did not fit as well. Since cumulative exposure and years since hire may be confounded, their interaction was examined. The interaction was not significant for any of the models. The same models were refitted excluding the largest exposure group (200+ ppm-years), but this did not significantly affect any

TABLE 8 Parameter estimates and model deviances for each of four models fitted to mean cumulative exposure per person-year for Delzell *et al.* (1995) study and comparison to parameter estimates from Delzell *et al.* analyses

Model	Parameter estimates	Standard error	Deviance	Parameter estimates from Delzell *et al.* study	p-value[1]
1) $RR = (1 + dose)^{\alpha}$	$\alpha = 0.2850$	$SE(\alpha) = 0.0976$	171.5	$\alpha = 0.2028$	0.39
2) $RR = 1 + \beta \cdot dose^{\alpha}$	$\alpha = 0.3999$ $\beta = 0.4558$	$SE(\alpha) = 0.2733$ $SE(\beta) = 0.8222$	172.0	$\alpha = 0.5000$[2] $\beta = 0.1293$	0.62
3) $RR = e^{\beta \cdot dose}$	$\beta = 0.0029$	$SE(\beta) = 0.0014$	176.7	$\beta = 0.0041$	0.38
4) $RR = 1 + \beta \cdot dose$	$\beta = 0.0099$	$SE(\beta) = 0.0065$	174.7	$\beta = 0.0068$	0.63

[1] p-value of likelihood ratio test of equality of parameters.

[2] For the Delzell *et al.* analysis, α was fixed at 0.5, and only β was estimated.

of the parameter estimates. The four models were also refitted allowing for different background rates for control and exposed populations. Different background rates might be necessary in occupational studies where lifetime non-exposed workers may differ fundamentally from exposed workers as a result of differences in jobs and work areas. Results of this analysis indicated that different background rates are not necessary for these data.

The parameter estimates obtained in the present analysis are also not significantly different from those presented in the Delzell *et al.* (1995) report. The differences in parameter estimates are likely due to the different levels used in the stratification variables. Table 8 compares the parameter estimates obtained in this analysis with those of the Delzell *et al.* (1995) report.

Cancer potency (TC_{01}):

The TC_{01}s were calculated for each model using the lifetable methods described above, and the resulting ambient occupational exposures per person-year were converted to environmental exposures by assuming that the exposures occurred for 8 hours per day, 240 days per year. This amounts to multiplying the TC_{01} by:

$$\frac{8 \text{ hours}}{24 \text{ hours}} \cdot \frac{240 \text{ days}}{365 \text{ days}}$$

To convert the ambient exposures from ppm to mg/m^3, the TC_{01}s are further multiplied by 2.21, the conversion factor for butadiene. The occupational and equivalent environmental TC_{01}s are presented in Table 9. Environmental TC_{01}s for each of the four models ranged from 1.4 to 4.3 mg/m^3. TC_{01}s calculated excluding the largest exposure group were slightly smaller, ranging from 0.6 to 1.6 mg/m^3, while those calculated on the basis of median exposures were similar, ranging from 0.4 to 5.0 mg/m^3.

TC_{01}s were also calculated using the parameter estimates from the Delzell *et al.* (1995) report and are compared with the TC_{01}s developed here in Table 9. They ranged from 3.1 to 14.3 mg/m^3.

TABLE 9 Carcinogenic potency estimates (TC_{01}s) for models fitted to mean cumulative exposure per person-year based on Delzell *et al.* (1995) study and comparison to estimates from Delzell *et al.* analyses

Model	Current analysis		Delzell *et al.* analysis
	Occupational TC_{01} (mg/m^3)	Environmental TC_{01} (mg/m^3)	Environmental TC_{01} (mg/m^3)
1) $RR = (1 + dose)^{\alpha}$	7.8	1.7	14.3
2) $RR = 1 + \beta \cdot dose^{\alpha}$	6.5	1.4	6.4
3) $RR = e^{\beta \cdot dose}$	19.8	4.3	3.1
4) $RR = 1 + \beta \cdot dose$	13.8	3.0	4.5

Lagged exposure analysis:

The same four models were fitted when exposures were lagged by 2, 5, 10, 15, 20 and 25 years. The resulting model fits are displayed in Table 10. Since the deviances are similar for each lag period, this analysis indicates that lagging exposures does not dramatically improve the fit of any of the four models. In fact, TC_{01}s for all four models and all lag periods ranged from 0.8 to 4.3 mg/m^3.

Validation study:

With respect to model validation, the p-values for the tests of equality of the parameters are displayed in Table 11. If the models were providing consistent fits between the two halves, the proportion of p-values less than the significance level of α would be α. The results of the simulation study indicate that the test is rejecting more often than would be expected if the models were providing the same fits to both halves of the data. For model 1, the test was rejected at a significance level of 1% in 7.4% of the runs, whereas a rejection rate of 1% of the runs would be expected if the model was fitting consistently. The results of this analysis reduce the confidence in the power of the models to predict leukemia mortality rates.

Summary

It is noteworthy that the choice of the exposure–response model does not have a large impact on the resulting TC_{01}; as indicated in Table 9, the values are similar, ranging from 1.4 to 4.3 mg/m^3. However, if a best model must be chosen, it would be model 1, owing to the smaller deviance (Table 8), the shape of the curve relative to the data in the low-dose region (Figure 2) and the fact that it has one fewer parameter than model 2, which provides a similar fit. The TC_{01} for model 1 is 1.7 mg/m^3.

It is difficult, though, to assess how well any of these models truly describes the data. It is noted that the plot in Figure 2 provides only a rough indication of the shape of the data, since each point on the plot is an average of data in many strata. The results of the validation study reduce confidence in the ability of the models to predict leukemia mortality.

The choice of exposure lag does not greatly improve the fit of any of the four models, and it does not affect the resulting TC_{01}. Including all lagged models, the range of TC_{01}s is still from 0.8 to 4.3 mg/m^3.

For comparison with these values, potency estimates were also calculated on the basis of the recent case–control study of styrene-butadiene rubber workers by Matanoski *et al.*

TABLE 10 Parameter estimates and model deviances for each of four lagged-exposure models fitted to median cumulative exposure per person-year

Model	Lag	Parameter estimates	Standard error	Deviance
1) $RR = (1 + dose)^{\alpha}$	None	$\alpha = 0.2850$	$SE(\alpha) = 0.0976$	171.5
	2 years	$\alpha = 0.2852$	$SE(\alpha) = 0.0982$	171.6
	5 years	$\alpha = 0.2883$	$SE(\alpha) = 0.0995$	171.6
	10 years	$\alpha = 0.3064$	$SE(\alpha) = 0.1034$	171.1
	15 years	$\alpha = 0.2955$	$SE(\alpha) = 0.1079$	172.4
	20 years	$\alpha = 0.2891$	$SE(\alpha) = 0.1141$	173.6
	25 years	$\alpha = 0.2898$	$SE(\alpha) = 0.1334$	175.4
2) $RR = 1 + \beta \cdot dose^{\alpha}$	None	$\alpha = 0.3999$	$SE(\alpha) = 0.2733$	172.0
		$\beta = 0.4557$	$SE(\beta) = 0.8219$	
	2 years	$\alpha = 0.3992$	$SE(\alpha) = 0.2738$	172.0
		$\beta = 0.4602$	$SE(\beta) = 0.8279$	
	5 years	$\alpha = 0.4024$	$SE(\alpha) = 0.2737$	172.0
		$\beta = 0.4647$	$SE(\beta) = 0.8288$	
	10 years	$\alpha = 0.4245$	$SE(\alpha) = 0.2755$	171.4
		$\beta = 0.4693$	$SE(\beta) = 0.8345$	
	15 years	$\alpha = 0.4835$	$SE(\alpha) = 0.3397$	172.6
		$\beta = 0.2878$	$SE(\beta) = 0.5846$	
	20 years	$\alpha = 0.4720$	$SE(\alpha) = 0.3558$	173.9
		$\beta = 0.3243$	$SE(\beta) = 0.6572$	
	25 years	$\alpha = 0.2960$	$SE(\alpha) = 0.2833$	175.3
		$\beta = 0.9293$	$SE(\beta) = 1.5710$	
3) $RR = e^{\beta \cdot dose}$	None	$\beta = 0.0029$	$SE(\beta) = 0.0014$	176.7
	2 years	$\beta = 0.0029$	$SE(\beta) = 0.0015$	176.8
	5 years	$\beta = 0.0031$	$SE(\beta) = 0.0015$	176.7
	10 years	$\beta = 0.0034$	$SE(\beta) = 0.0016$	176.4
	15 years	$\beta = 0.0035$	$SE(\beta) = 0.0018$	177.0
	20 years	$\beta = 0.0033$	$SE(\beta) = 0.0022$	178.2
	25 years	$\beta = 0.0033$	$SE(\beta) = 0.0022$	178.2
4) $RR = 1 + \beta \cdot dose$	None	$\beta = 0.0099$	$SE(\beta) = 0.0065$	174.7
	2 years	$\beta = 0.0102$	$SE(\beta) = 0.0067$	174.7
	5 years	$\beta = 0.0109$	$SE(\beta) = 0.0072$	174.6
	10 years	$\beta = 0.0137$	$SE(\beta) = 0.0089$	173.8
	15 years	$\beta = 0.0158$	$SE(\beta) = 0.0106$	174.1
	20 years	$\beta = 0.0179$	$SE(\beta) = 0.0129$	175.7
	25 years	$\beta = 0.0179$	$SE(\beta) = 0.0129$	175.7

(1997). Although workers were from plants subsumed in the Delzell *et al.* (1995) study, exposure was independently characterized. Treating the odds ratio presented by these authors as a rate ratio (since leukemia is a rare disease) and using their model and parameter estimates as well as the same lifetable methods described above, the TC_{01} for environmental exposure was calculated to be 0.4 mg/m³. It is reassuring, therefore, that this value is only slightly lower than the estimates derived on the basis of the Delzell *et al.* (1995) cohort study data.

TABLE 11 Model validation p-values for Delzell *et al.* (1995) study

Model	Proportion of p-values[1] that are		
	less than 0.01	less than 0.05	less than 0.1
1) RR = (1 + dose)$^{\alpha}$	0.074	0.167	0.252
2) RR = 1 + β·dose$^{\alpha}$	0.084	0.19	0.286
3) RR = $e^{\beta \cdot dose}$	0.08	0.188	0.264
4) RR = 1 + β·dose	0.103	0.214	0.303

[1] p-value of likelihood ratio test of equality of parameters fitted to each half of the data.

3.3.3.1.2 Estimated potency based on data from studies in experimental animals

As described in Section 3.3.2, butadiene induced an increase in the incidence of tumours at multiple sites in both B6C3F$_1$ mice (liver, lung, Harderian gland, mammary gland, ovaries, forestomach, Zymbal gland and kidney, along with malignant lymphomas, histiocytic sarcomas and cardiac hemangiosarcomas) and Sprague-Dawley rats (mammary gland, thyroid gland, uterus, Zymbal gland, pancreas and testes). As discussed above, consistent with the species differences in metabolism, mice were much more sensitive to butadiene-induced cancer than were rats for the strains investigated. Based on data available (i.e., evidence from genotoxicity studies that butadiene and its metabolites are active in both species), this difference in sensitivity is quantitative rather than qualitative and is related to the greater amounts of putatively active metabolites formed in mice compared with rats. In addition, the different profiles of tumours observed in the two species may be related to differential roles of the epoxide metabolites in the induction of the various tumours; i.e., the diepoxide may be more critical to tumour induction in mice than is EB (since it was reported recently that formation of DEB increased with level of exposure to butadiene in mice but not in rats; Thornton-Manning *et al.*, 1998), while the monoepoxide or monoepoxide diol may be more important in rats.

The relevance for extrapolation to humans of exposure–response for some of the types of tumours observed in rodents has been questioned. For example, Irons *et al.* (1989) hypothesized that the thymic lymphoma/leukemia induced in B6C3F$_1$ mice may be related to the presence of an endogenous ecotropic retrovirus, as a much lower incidence was observed in Swiss mice that do not possess this retrovirus (although the incidence was significantly elevated compared with controls). Therefore, although the hematopoietic system is a target for the induction of cancer by butadiene in humans, the observed exposure–response relationship for this endpoint is not considered appropriate for quantitative extrapolation to humans — on the basis that this retrovirus is not present in humans and its presence in B6C3F$_1$ mice renders this strain quite susceptible to induction of lymphoma — although the relevant information is included for comparative purposes.

It has also been suggested that the tumours observed in the study in rats (i.e., mammary gland, thyroid gland, pancreas, uterus and testes) and some of the tumours induced in mice (i.e., ovaries and mammary gland) may be mediated through effects on the endocrine system. Indeed, tumours at these sites are often associated with disruption of hormonally mediated functions. In addition, non-neoplastic or pre-neoplastic effects, including atrophy, degeneration and hyperplasia, have also been observed in mice exposed subchronically to butadiene. However, the mechanism by which butadiene induces tumours at these sites has not yet been adequately investigated; i.e., it has not been established

whether these tumours are induced via a mechanism for which there may be a threshold of exposure (e.g., through induction of hormonally mediated effects), although the possibility is recognized. In addition, the results of *in vivo* genotoxicity assays indicate that butadiene or its metabolites induce genetic effects in the reproductive organs of multiple strains of mice.

Based on these considerations, estimates of carcinogenic potency were calculated on the basis of the malignant lymphomas, histiocytic sarcomas, cardiac hemangiosarcomas, alveolar/bronchiolar adenomas or carcinomas, hepatocellular adenomas or carcinomas, squamous cell papillomas or carcinomas of the forestomach, adenomas or carcinomas of the Harderian gland, granulosa cell tumours of the ovaries and adenoacanthomas, carcinomas or malignant mixed tumours of the mammary gland observed in $B6C3F_1$ mice in the chronic bioassay conducted by the NTP (1993) and the mammary gland tumours, pancreatic exocrine adenomas, Leydig cell tumours, Zymbal gland carcinomas, thyroid follicular cell adenomas or carcinomas and uterine sarcomas in Sprague-Dawley rats reported by Hazleton Laboratories Europe Ltd. (1981a). (The tumour incidence data for each of the sites considered are presented in Table 3.) It is noted that the characterization of exposure–response is much better in the study in mice (which involved five closely spaced exposure levels) than in the bioassay in rats (in which only two more widely spaced exposure levels were used, the higher of which was likely above the level of metabolic saturation). (N.B.: Although there were also increased incidences of tumours at several sites in $B6C3F_1$ mice in the "stop-exposure" study conducted by the NTP [1993], only TC_{05}s determined on the basis of the 2-year study were included, as the latter study provides better information for characterization of exposure–response in mice following long-term exposure [i.e., more exposure levels for up to 2 years].)

In the NTP study, mice were exposed to 0, 6.25, 20, 62.5, 200 or 625 ppm (0, 13.8, 44.2, 138, 442 or 1383 mg/m^3) butadiene for 6 hours per day, 5 days per week, for 103 weeks. Survival of mice decreased with increasing exposure concentration; therefore, to minimize the effect of the high mortality rate, the poly-3 adjusted data presented in the NTP (1993) report were used in these calculations. For some tumour types, the adjusted data still demonstrated downward curvature at the highest concentration. In these cases, the high-exposure group was excluded in the determination of the TC_{05}. The TC_{05}s were calculated for these endpoints by first fitting a multistage model to the data. The multistage model is given by:

$$P(d) = 1 - e^{-q_0 - q_1 d - \ldots - q_k d^k}$$

where d is dose, k is the number of dose groups in the study minus one, $P(d)$ is the probability of the animal developing a tumour at dose d and $q_i > 0$, $i = 1,\ldots, k$ are parameters to be estimated.

The models were fitted using GLOBAL82 (Howe and Crump, 1982), and a chi-square lack of fit test was performed for each model fit. The degrees of freedom for this test are equal to k minus the number of q_i's whose estimates are non-zero. A p-value less than 0.05 indicates a significant lack of fit. Results from the model fitting are displayed in Table 12. Plots of the data and the fitted models are shown in Figure 3.

TC_{05}s were determined as the doses D (in mg/m^3) that satisfy

$$\frac{P(D) - P(0)}{1 - P(0)} = 0.05$$

and then adjusted by multiplying by:

$$\frac{6 \text{ h/day}}{24 \text{ h/day}} \cdot \frac{5 \text{ days/week}}{7 \text{ days/week}} \cdot \frac{w \text{ weeks}}{104 \text{ weeks}} \cdot \left(\frac{w \text{ weeks}}{104 \text{ weeks}}\right)^2$$

Table 12 Carcinogenic potency estimates (TC_{05}s)[1] of butadiene based on results of bioassays in experimental animals

Tumour type	Males					Females				
	TC_{05} (mg/m³)	95% LCL (mg/m³)	χ^2 [2]	df[3]	p[4]	TC_{05} (mg/m³)	95% LCL (mg/m³)	χ^2	df	p
Mice (from NTP, 1993)										
Alveolar/bronchiolar adenomas or carcinomas	2.4	1.4	1.0	3	0.79	5.2	3.2	9.1	4	0.06
Histiocytic sarcomas	12	8.4	7.6	5	0.18	21	12	5.4	4	0.25
Cardiac hemangiosarcomas	14	6.4	0.34	3	0.95	7.6	5.2	18	4	0.00
Forestomach squamous cell papillomas or carcinomas	29	13	6.1	3	0.11	14	8.1	4.3	4	0.36
Ovarian granulosa cell tumours	–	–	–	–	–	6.7	4.4	5.0	3	0.17
Mammary gland adenoacanthomas, carcinomas or malignant mixed tumours	–	–	–	–	–	6.7	4.9	13	4	0.01
Hepatocellular adenomas or carcinomas	3.2	1.9	2.8	2	0.24	5.4	3.2	0.8	3	0.85
Harderian gland adenomas or carcinomas	2.3	1.7	0.5	2	0.77	4.7	2.7	1.5	2	0.47
Malignant lymphomas[5]	99	23	3.3	3	0.35	23	6.9	3.9	3	0.27
Rats (from Hazleton Laboratories Europe Ltd., 1981a)										
Mammary gland adenomas or carcinomas	–	–	–	–	–	6.7	4.7	0	0	–
Pancreatic exocrine adenomas	597	316	1.1	1	0.29	–	–	–	–	–
Leydig cell tumours	161	96	0	1	–	–	–	–	–	–
Thyroid follicular cell adenomas or carcinomas	–	–	–	–	–	142	113	0	1	–
Uterine sarcomas	–	–	–	–	–	189	113	0	0	–
Zymbal gland carcinomas	1023	905	1	1	0.32	4872	766	0.06	2	0.97

[1] Values have been adjusted for lifetime exposure.

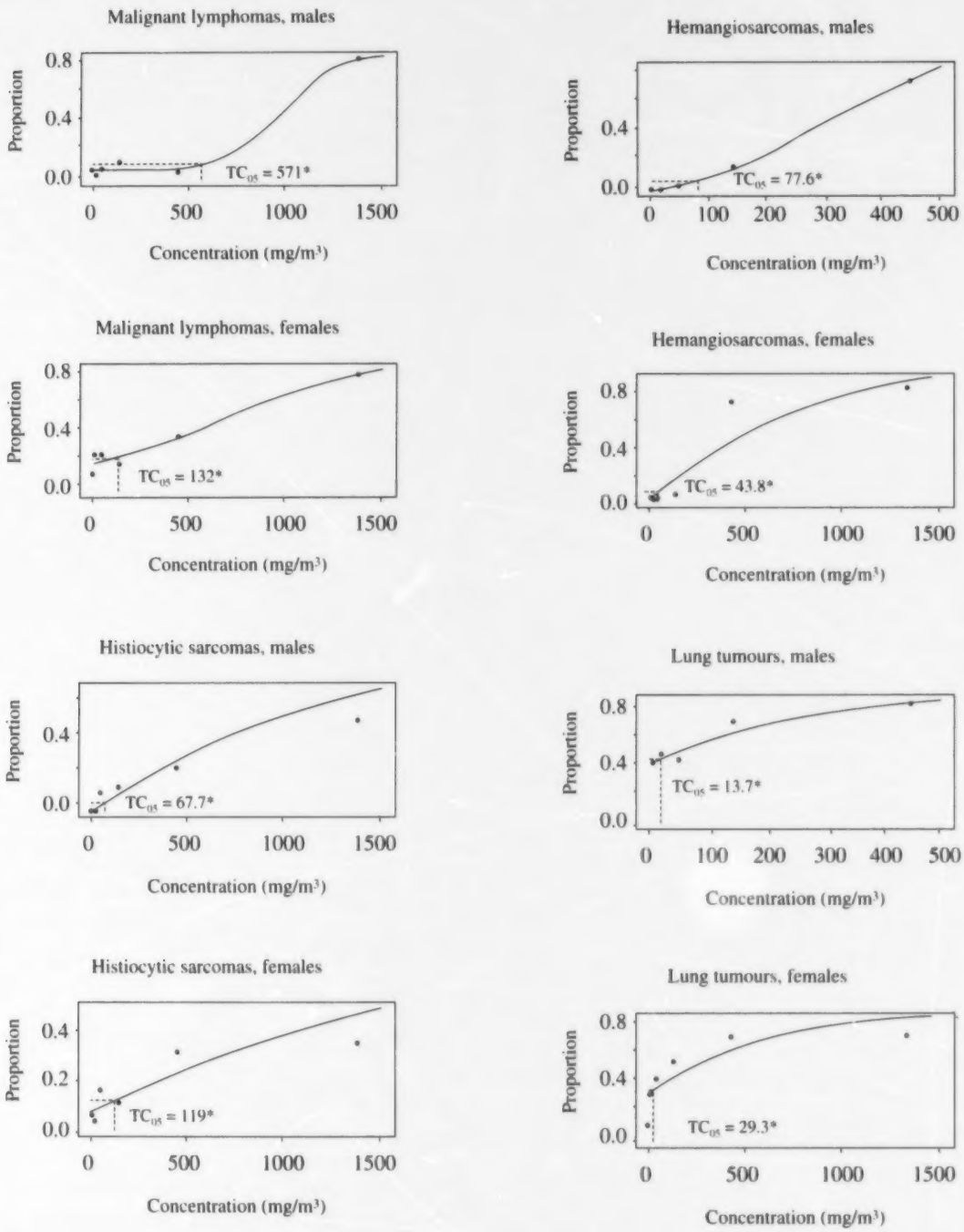
[2] Chi-squared goodness of fit statistic.

[3] Degrees of freedom.

[4] p-value of goodness of fit test (p-value < 0.05 indicates significant lack of fit).

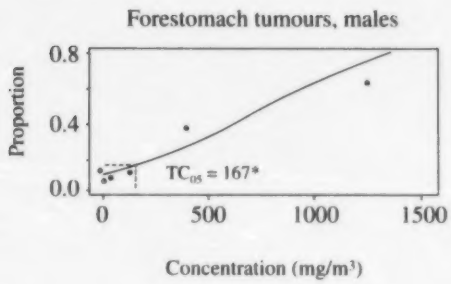
[5] Values for malignant lymphomas presented here only for comparison; potency estimates for these tumours not considered relevant to humans due to the greater sensitivity of these mice to induction of this effect associated with the presence of an endogenous retrovirus.

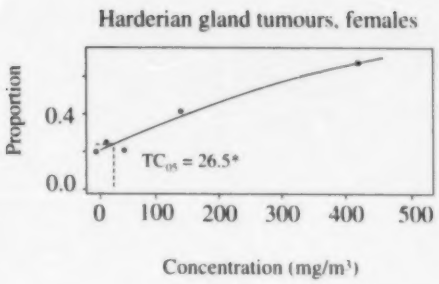
FIGURE 3 Exposure–response analysis for butadiene-induced tumours in mice

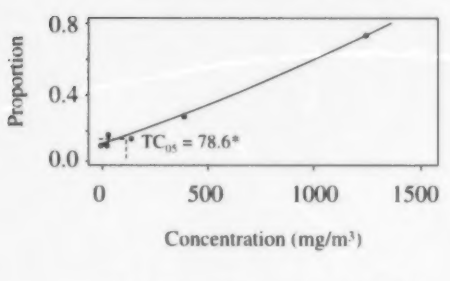


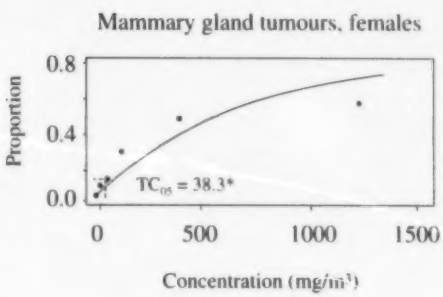
*TC_{05} unadjusted for lifetime dosing

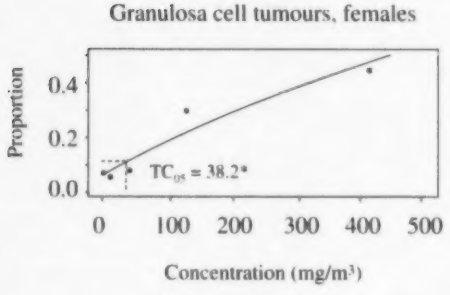
FIGURE 3 (continued)

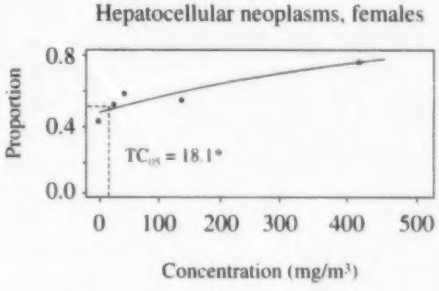


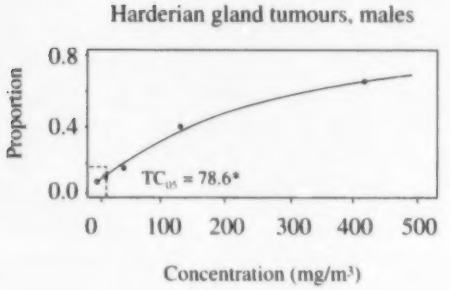


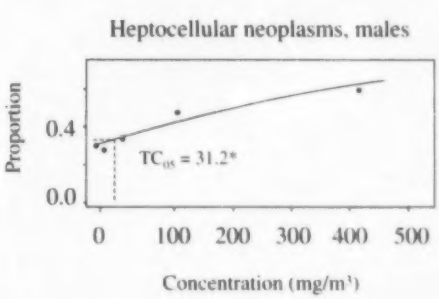












*TC_{05} unadjusted for lifetime dosing

where, in the first term, which amortizes the dose to be constant over the lifetime of a mouse, w is the duration of the experiment (103 weeks). The second factor was suggested by Peto *et al.* (1984) and corrects for an experiment length that is unequal to the standard lifetime. Since tumours develop much more rapidly later in life, a greater than linear increase in the tumour rate is expected when animals are observed for tumours longer than their standard lifetime (or the reverse when animals are observed for a period shorter than their standard lifetime). (N.B.: Application of this factor does not impact greatly on the final values, since it is very close to one.) The selected TC_{05} values for this study and their 95% lower confidence limits (LCLs) are presented in Table 12 and range from 2.3 mg/m^3 (95% LCL = 1.7 mg/m^3) or 1.1 ppm (95% LCL = 0.79 ppm) for Harderian gland tumours in males to 99 mg/m^3 (95% LCL = 23 mg/m^3) or 45 ppm (95% LCL = 10 ppm) for malignant lymphomas in males.

Estimates of carcinogenic potency were also calculated based on the results of the bioassay in Sprague-Dawley rats (Hazleton Laboratories Europe Ltd., 1981a). In this study, rats were exposed to 0, 1000 or 8000 ppm (0, 2212 or 17 696 mg/m^3) for 6 hours per day, 5 days per week, for 105 (males) or 111 (females) weeks. A high mortality rate was observed at the higher concentration; therefore, this exposure group was excluded from the analysis, except for the potency estimates for pancreatic exocrine adenomas in males (for this endpoint, exclusion of the high-exposure group would have resulted in the exposure–response relationship curving downwards). As for mice, a multistage model was fit to the data for rats using GLOBAL82 and adjusted to account for study duration (w) by multiplying by:

$$\frac{6\text{ h/day}}{24\text{ h/day}} \cdot \frac{5\text{ days/week}}{7\text{ days/week}} \cdot \frac{w\text{ weeks}}{104\text{ weeks}} \cdot \left(\frac{w\text{ weeks}}{104\text{ weeks}}\right)^2$$

where the duration of the experiment was 105 weeks for males and 111 weeks for females.

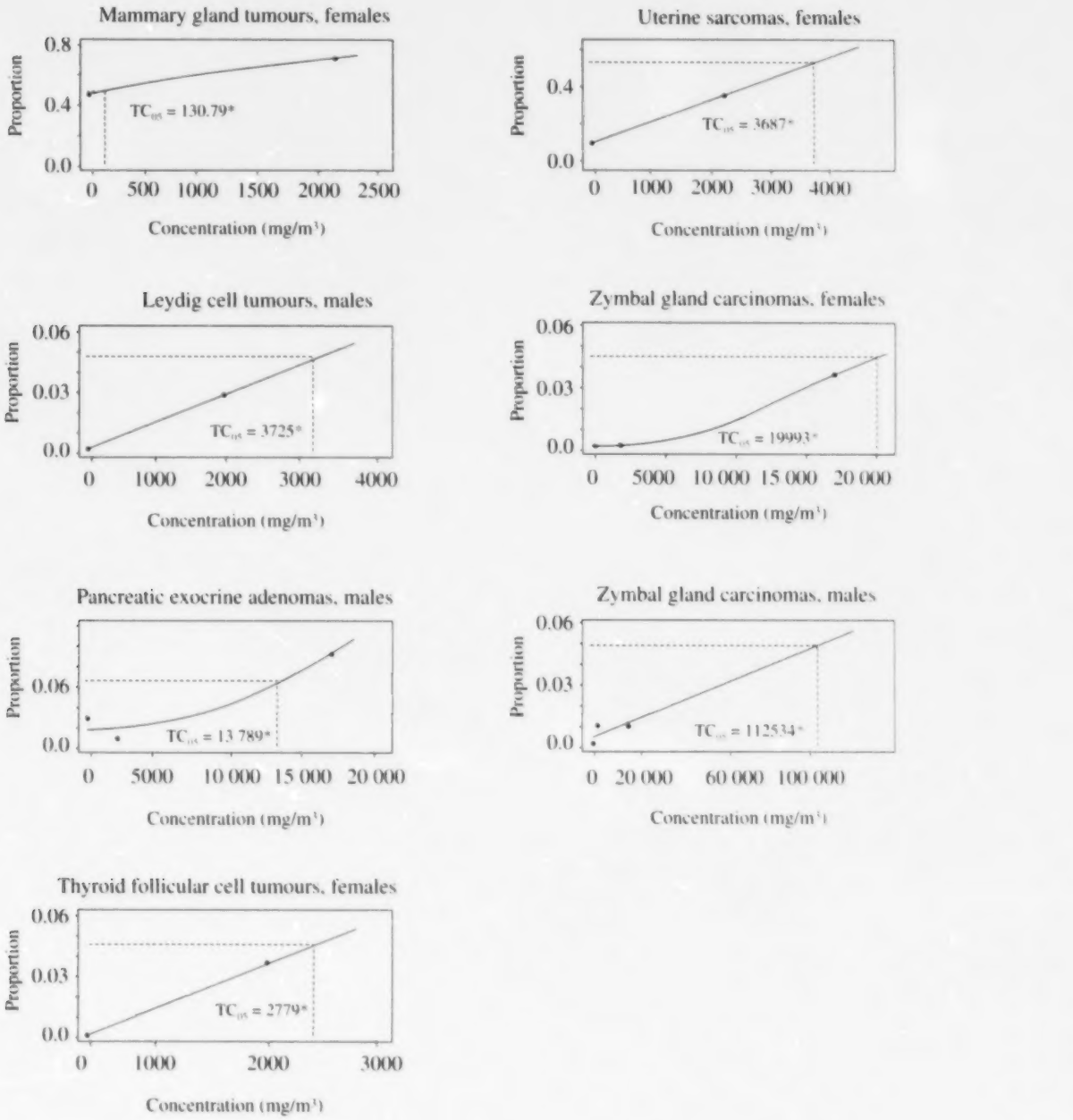
The exposure–response curves and estimated adjusted TC_{05} values based on this study in rats are presented in Figure 4 and Table 12, respectively. The concentrations of butadiene estimated to be associated with a 5% increased incidence of tumours ranged from 6.7 mg/m^3 (95% LCL = 4.7 mg/m^3) or 3.0 ppm (95% LCL = 2.1 ppm) to 4872 mg/m^3 (95% LCL = 766 mg/m^3) or 2203 ppm (95% LCL = 346 ppm) for tumours of the mammary gland and Zymbal gland in female rats, respectively. Although the available data for analysis of exposure–response were more limited for rats than for mice, it is interesting to note the similarity in estimates of potency for mammary gland tumours (i.e., 6.7 mg/m^3 in both species).

Based on modelling (using THC; Howe, 1995a) of the incidence of micronucleated polychromatic erythrocytes in B6C3F$_1$ mice exposed to butadiene for up to 15 months in the NTP bioassay, BMC_{05}s for somatic cell mutations were very similar to the lower end of the range of the TC_{05}s for tumour induction.

3.3.3.2 Non-neoplastic effects

There have been recent attempts to quantitatively estimate risk of heritable genetic damage in humans based on a parallelogram approach and data on male-mediated heritable translocations and bone marrow micronuclei in mice and chromosomal aberrations in lymphocytes of exposed workers (Pacchierotti *et al.*, 1998b). In view, however, of the reported ovarian atrophy due to reduction of primordial follicles (to a degree that would preclude reproduction) following chronic exposure of mice to concentrations of butadiene considerably lower than those associated with adverse effects on the testes, investigation of the response of female germ cells in mice to butadiene is desirable, since this may well be the most sensitive endpoint for development of quantitative estimates of heritable damage. (Determination of putatively toxic metabolites in the ovaries of butadiene-exposed female mice would also be informative.) For this reason, quantitation of exposure–response

FIGURE 4 Exposure--response analysis for butadiene-induced tumours in rats



*TC_{05} unadjusted for lifetime dosing

for heritable genetic damage is not presented here. However, in view of the apparent greater sensitivity of the reproductive organs in female mice, a benchmark concentration was derived for non-neoplastic effects in the ovary, which is considerably more protective than that for male-mediated heritable damage developed by Pacchierotti *et al.* (1998b). (Although the relative role of butadiene in the induction of the observed atrophy in mice in the NTP study is unclear, as discussed in Section 3.3.2.2, information currently available is not considered a sufficient basis upon which to dismiss this endpoint as being inappropriate for quantification of exposure–response. However, this uncertainty should be kept in mind in the interpretation or application of the BMC_{05}s derived below.)

Hematotoxicity is considered to be a critical effect associated with exposure to butadiene. Although the hematopoietic system appears to be a target for butadiene-induced cancer in humans, available data on the potential non-neoplastic effects on this system are inadequate for quantitation of exposure–response. However, since statistically significant changes were observed in mice only at concentrations greater than those that induced other toxic effects, and since benchmark concentrations derived for effects on the blood are greater than those for these other effects, quantitation of the exposure–response for hematological effects has not been presented here.

Ovarian atrophy was observed in both long-term NTP (1984, 1993) bioassays in mice and a subchronic study (Bevan *et al.*, 1996). Although limited, available data indicate that rats are less sensitive to induction of this effect, which may, again, be a consequence of interspecies variations in metabolism. Therefore, although additional research into the etiology of the observed ovarian atrophy in mice would be desirable, the data from the later NTP study are considered most appropriate for characterization of exposure–response (i.e., development of a BMC_{05}). In this investigation, the incidence of atrophy of the ovaries was significantly increased in an exposure-related manner at all concentrations tested (i.e., ≥ 6.25 ppm [≥13.8 mg/m^3]). The severity of this effect also increased with exposure (see Table 13).

The exposure–response relationship for ovarian atrophy from this study was quantified by fitting the following model to the dose–response data (Howe, 1995b):

$$P(d) = \begin{cases} q_0 & \text{if } d \le d_0 \\ q_0 + (1-q_0)\cdot\left[1-e^{-q_1(d-d_0)-\ldots-q_k(d-d_0)^k}\right] & \text{if } d > d_0 \end{cases}$$

where d is dose, k is the number of dose groups in the study minus one, $P(d)$ is the probability of the animal developing the effect at dose d and $q_i > 0$, $i = 1, \ldots, k$ and d_0 are parameters to be estimated. The models were fit using THRESH (Howe, 1995b), and the BMC_{05}s were calculated as the dose D that satisfies:

$$\frac{P(D) - P(0)}{1 - P(0)} = 0.05$$

A chi-square lack of fit test was performed for each of the model fits. The degrees of freedom for this test are equal to k minus the number of q_i's whose estimates are non-zero. A p-value less than 0.05 indicates a significant lack of fit.

The BMC_{05} was then amortized to be constant over the standard life of a mouse by multiplying by:

$$\frac{6 \text{ hours/day}}{24 \text{ hours/day}} \cdot \frac{5 \text{ days/week}}{7 \text{ days/week}}$$

Resulting BMC_{05}s and lack of fit information for all models fit are displayed in Table 14.

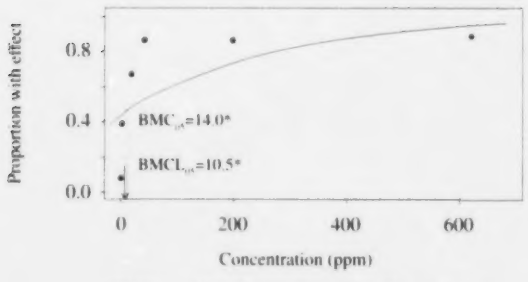
The model fitted to all six exposure groups exhibited a significant lack of fit, likely due to the fact that the curve rises sharply and then plateaus at the three highest exposure groups. Plots of the data and fitted model are displayed

TABLE 13 Incidence and severity of ovarian atrophy observed in 2-year bioassay in mice (NTP, 1993)

Exposure level (ppm)	Number of animals examined	All severities	Minimal	Mild	Moderate	Marked
0	49	4	1	2	1	0
6.25	49	19	0	15	4	0
20	48	32	1	23	8	0
62.5	50	42	3	18	21	0
200	50	43	0	9	34	0
625	79	69	0	19	47	3

in Figure 5. Since a good fit in the range of the BMC_{05} (in the vicinity of 6.25 ppm [13.8 mg/m^3]) is desired, the model was refitted omitting the two highest exposure groups. This model again indicates a marginal lack of fit. The graph of this model (Figure 6) indicates that this model provides a reasonable visual fit to the data, but the resulting BMC_{05} is uncertain due to lack of fit of the model.

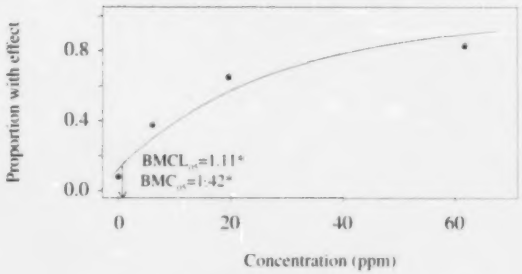
FIGURE 5 Exposure–response analysis for ovarian atrophy in mice



*BMC_{05} and $BMCL_{05}$ unadjusted for lifetime dosing

The BMC_{05} for the model excluding the two highest dose groups was calculated to be 0.57 mg/m^3, with a 95% LCL of 0.44 mg/m^3.

FIGURE 6 Exposure–response analysis for ovarian atrophy in mice, excluding two highest dose groups

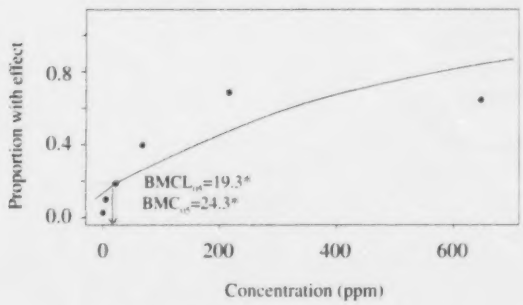


*BMC_{05} and $BMCL_{05}$ unadjusted for lifetime dosing

TABLE 14 Benchmark concentrations for ovarian atrophy

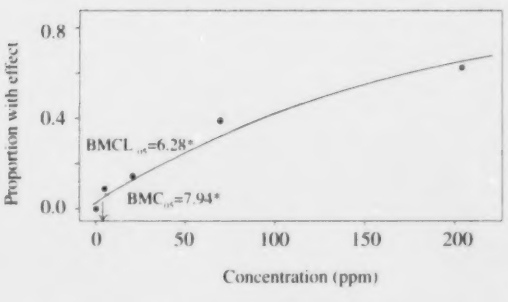
Ovarian atrophy	BMC_{05} (ppm)	95% LCL on BMC_{05} (ppm)	BMC_{05} (mg/m^3)	95% LCL on BMC_{05} (mg/m^3)	Chi-square	df	p-value
All severities	2.5	1.9	5.6	4.1	61	4	0.00
All severities, excluding top two dose groups	0.25	0.20	0.57	0.44	7.0	2	0.03
Moderate/marked severity	4.3	3.4	9.6	7.6	37.1	4	0.00
Moderate/marked severity, excluding top dose group	1.4	1.1	3.1	2.5	2.2	3	0.55

FIGURE 7 Exposure–response analysis for moderate/marked ovarian atrophy



*BMC_{05} and $BMCL_{05}$ unadjusted for lifetime dosing

FIGURE 8 Exposure–response analysis for moderate/marked ovarian atrophy, excluding high-dose group



*BMC_{05} and $BMCL_{05}$ unadjusted for lifetime dosing

If only those animals that had moderate or marked ovarian atrophy from all exposure groups were included, the resulting BMC_{05} would be 9.6 mg/m^3 (95% LCL = 7.6 mg/m^3), although there is again a significant lack of fit (Figure 7). If the highest exposure group is excluded, the BMC_{05} for moderate or marked ovarian atrophy becomes 3.1 mg/m^3, with a 95% LCL of 2.5 mg/m^3 (Figure 8).

3.3.4 Human health risk characterization

Butadiene is released to air in Canada from both industrial point sources and more dispersive, non-point sources, the latter due to its production primarily during incomplete combustion. Intake for the general population in Canada is primarily from air, with intake from other media likely being negligible in comparison. The focus of the human health risk characterization is, therefore, the general population exposed in outdoor and indoor air in the general environment and those exposed through air in the vicinity of industrial point sources.

For compounds such as butadiene, where data are sufficient to support a plausible mode of action for induction of tumours by direct interaction with genetic material, estimates of exposure are compared with quantitative estimates of cancer potency (Exposure Potency Index or EPI) to characterize risk and provide guidance

in establishing priorities for further action (i.e., analysis of options to reduce exposure) under CEPA (Health Canada, 1994).

Tumorigenic concentrations were calculated on the basis of data from both epidemiological studies and investigations in experimental animals. For the critical epidemiological investigation (Delzell *et al.*, 1995), a TC_{01} (i.e., the concentration associated with a 1% increase in mortality due to leukemia) was considered the appropriate measure of carcinogenic potency, since the majority of the observable data fell within this range. Although four different mathematical models were considered, the TC_{01} generated by the model with the best fit was 1.7 mg/m^3.

Quantitative estimates of carcinogenic potency derived on the basis of data in experimental animals were calculated as TC_{05}s (i.e., the concentration associated with a 5% increase in tumour incidence). Based on the 2-year bioassay in mice (NTP, 1993), TC_{05}s ranged from 2.3 mg/m^3 (95% LCL = 1.7 mg/m^3) to 99 mg/m^3 (95% LCL = 23 mg/m^3). The TC_{05}s derived on the basis of the more limited study in rats (Hazleton Laboratories Europe Ltd., 1981a) ranged from 6.7 mg/m^3 (95% LCL = 4.7 mg/m^3) to 4872 mg/m^3 (95% LCL = 766 mg/m^3).

The values derived on the basis of studies in humans are preferred as the basis for comparison with estimates of exposure to characterize risk. While there are a number of uncertainties in the use of the epidemiological data for both hazard evaluation and exposure–response analyses (Section 3.3.5), these are likely far less than uncertainties associated with interspecies extrapolation. Moreover, estimated potency for humans is similar to that developed on the basis of the cancer bioassays in experimental animals. (Indeed, although in an area of the exposure–response curve where data were more sparse, it is noteworthy that TC_{05}s calculated on the basis of epidemiological data [as opposed to the TC_{01}s presented above] are within the range of values derived from the studies in rodents.)

Based on the estimates of exposure presented above (Section 3.3.1), 95% of the population is exposed to concentrations of butadiene in outdoor air of 1.0 µg/m^3 or less. For the proportion of the general population that is regularly exposed to higher concentrations of

COMPARISON OF ESTIMATES OF CARCINOGENIC POTENCY WITH EXPOSURE LEVELS

Exposure	Potency (TC_{01} or TC_{05})	Margin between effect level and exposure*	Priority for further action*
		Exposure Potency Index (EPI)*	
1.0 µg/m^3 (95th percentile for all sites in Canada)	1.7 mg/m^3 (TC_{01} for leukemia in humans)	1700	Moderate
		5.9×10^{-4}	
	2.3 mg/m^3 (TC_{05} for most sensitive tumour site in mice [Harderian gland])	2300	High
		4.3×10^{-4}	
	1.7 mg/m^3 (95% LCL of TC_{05} for most sensitive tumour site in mice)	1700	High
		5.9×10^{-4}	

COMPARISON OF ESTIMATES OF CARCINOGENIC POTENCY WITH EXPOSURE LEVELS (continued)

Exposure	Potency (TC_{01} or TC_{05})	Margin between effect level and exposure*	Priority for further action*
		Exposure Potency Index (EPI)*	
	6.7 mg/m³ (TC_{05} for most sensitive tumour site in rats [mammary gland])	6700	Moderate
		1.5×10^{-4}	
	4.7 mg/m³ (95% LCL of TC_{05} for most sensitive tumour site in rats)	4700	High
		2.1×10^{-4}	
1.3 µg/m³ (95th percentile for reasonable worst-case scenario)	1.7 mg/m³ (TC_{01} for leukemia in humans)	1300	Moderate
		7.6×10^{-4}	
	2.3 mg/m³ (TC_{05} for most sensitive tumour site in mice [Harderian gland])	1800	High
		5.7×10^{-4}	
	1.7 mg/m³ (95% LCL of TC_{05} for most sensitive tumour site in mice)	1300	High
		7.6×10^{-4}	
	6.7 mg/m³ (TC_{05} for most sensitive tumour site in rats [mammary gland])	5200	Moderate
		1.9×10^{-4}	
	4.7 mg/m³ (95% LCL of TC_{05} for most sensitive tumour site in rats)	3600	High
		2.8×10^{-4}	
6.4 µg/m³ (95th percentile for area affected by industrial point source)	1.7 mg/m³ (TC_{01} for leukemia in humans)	270	High
		3.8×10^{-3}	
	2.3 mg/m³ (TC_{05} for most sensitive tumour site in mice [Harderian gland])	360	High
		2.8×10^{-3}	
	1.7 mg/m³ (95% LCL of TC_{05} for most sensitive tumour site in mice)	270	High
		3.8×10^{-3}	
	6.7 mg/m³ (TC_{05} for most sensitive tumour site in rats [mammary gland])	1000	High
		9.6×10^{-4}	

COMPARISON OF ESTIMATES OF CARCINOGENIC POTENCY WITH EXPOSURE LEVELS (continued)

Exposure	Potency (TC_{01} or TC_{05})	Margin between effect level and exposure*	Priority for further action*
		Exposure Potency Index (EPI)*	
	4.7 mg/m³ (95% LCL of TC_{05} for most sensitive tumour site in rats)	730	High
		1.4×10^{-3}	

* For EPIs calculated on the basis of a TC_{01} derived from epidemiological data, the priority for investigation of options to reduce exposure is considered to be high, moderate or low if the EPI values are determined to be 1×10^{-3} or greater, between 1×10^{-3} and 1×10^{-5} or less than 1×10^{-5}, respectively. If EPIs are calculated on the basis of a TC_{05} derived from data in laboratory animals, the priority for investigation of options to reduce exposure is considered to be high, moderate or low if the EPI values are determined to be 2.0×10^{-4} or greater, between 2.0×10^{-4} and 2.0×10^{-6} or less than 2.0×10^{-6}, respectively.

butadiene in urban areas (i.e., the "reasonable worst-case scenario"), the 95th percentile of the distribution of concentrations is 1.3 µg/m³. In the only area of Canada identified as having an industrial point source, the 95th percentile of the distribution of concentrations is 6.4 µg/m³.

The margins between carcinogenic potency and estimated exposure for the general population (including ambient and reasonable worst case) and those in the vicinity of a point source are presented in the table above. Based on these margins, the priority for investigation of options to reduce exposure of the general population exposed in the ambient environment is considered to be moderate to high, while that for those in the vicinity of industrial point sources is considered to be high.

In view of the relative potency of butadiene to induce some non-cancer effects, these endpoints are also important in risk characterization. As presented above, a benchmark concentration (BMC_{05}) of 0.57 mg/m³ (95% LCL = 0.44 mg/m³) was derived on the basis of data for the incidence of ovarian atrophy of all severities (i.e., female reproductive toxicity) in mice exposed to butadiene for up to 2 years (NTP, 1993). And while there is uncertainty about the relevance of the ovarian atrophy observed in mice for humans (Section 3.3.5), the BMC_{05} is slightly less than the lower end of the range of estimates of cancer potency based on the incidence of tumours in the same study in mice, as well as the TC_{05} for cancer based on the epidemiological data. The mode of induction of ovarian atrophy is unknown. However, if it is (reasonably) assumed that the mode of action is related to that by which tumours are induced (i.e., direct interaction with genetic material), the priority for further action, based on the margin between estimated potency and exposure, is considered to be high. It should be noted, though, that even if the mode of induction of ovarian atrophy does not involve direct interaction with genetic material, the margin between exposure and effect level (i.e., for which a tolerable concentration is normally developed) is still inadequate — i.e., exposure levels in Canada are 90–570 times lower than the benchmark concentration, as presented below. Therefore, the priority for further action (i.e., priority for investigation of options to reduce exposure), based on this effect, is considered to be high.

Based on comparison of estimated exposure with the potency to induce leukemia in humans and cancer and non-cancer effects

COMPARISON OF ESTIMATES OF POTENCY FOR NON-CANCER EFFECTS WITH EXPOSURE LEVELS

Exposure	Potency (BMC_{05})	Margin between effect level and exposure*	Priority for further action*
		Exposure Potency Index (EPI)*	
1.0 µg/m³ (95th percentile for all sites in Canada)	0.57 mg/m³ (BMC_{05} for ovarian atrophy in mice)	570	High
		1.8×10^{-3}	
	0.44 mg/m³ (95% LCL of BMC_{05} for ovarian atrophy in mice)	440	High
		2.3×10^{-3}	
1.3 µg/m³ (95th percentile for reasonable worst-case scenario)	0.57 mg/m³ (BMC_{05} for ovarian atrophy in mice)	440	High
		2.3×10^{-3}	
	0.44 mg/m³ (95% LCL of BMC_{05} for ovarian atrophy in mice)	340	High
		3.0×10^{-3}	
6.4 µg/m³ (95th percentile for area affected by industrial point source)	0.57 mg/m³ (BMC_{05} for ovarian atrophy in mice)	90	High
		1.1×10^{-2}	
	0.44 mg/m³ (95% LCL of BMC_{05} for ovarian atrophy in mice)	70	High
		1.5×10^{-2}	

* If mode of action involves interaction with genetic material.

in experimental animals, and taking into consideration the degree of confidence in the database upon which the quantitative measures of toxicity were based, the overall priority for investigation of options to reduce exposure to butadiene in the general environment in Canada, based solely on potential adverse health effects, is considered to be moderate to high.

3.3.5 *Uncertainties and degree of confidence in human health risk characterization*

There is a high degree of certainty that butadiene is being released to ambient air in Canada in significant amounts in vehicular exhaust. There is a moderate degree of certainty that exhaust emissions of butadiene are lower in well-maintained vehicles equipped with catalytic converters than in older non-catalyst-equipped vehicles, and that evaporative emissions during refuelling and vehicle operation contribute less to concentrations of butadiene in ambient air than do emissions in vehicular exhaust.

There is a moderate degree of certainty that butadiene is not being released to the Canadian environment in significant amounts from industrial activities in Canada, as only a single major point source (i.e., in Sarnia, Ontario) of discharge to the atmosphere has been identified. Although there is some uncertainty that the available measurements of butadiene in samples taken over a few days in the vicinity of this source are representative of population exposure over the long term, since the samples were taken at distances of up to a few kilometres from the source, there is a moderate degree of certainty that a segment of the population would be exposed to the measured concentrations. There

is a high degree of certainty that populations in rural areas are exposed to lower concentrations of butadiene in ambient air than are communities in more densely populated areas.

Available data on concentrations of butadiene in ambient air in Canada are quite extensive. A large proportion of the numerous samples from several sampling sites across the country contained concentrations of butadiene above the level of detection. Therefore, there is a high degree of certainty in the estimations of exposure to butadiene via ambient air.

The most limiting aspect of the exposure assessment is the lack of sufficient data on the concentrations of butadiene in indoor air. This is an important shortcoming, since humans spend significantly greater time in indoor environments than outdoors. In the absence of indoor sources, it is reasonably certain that concentrations of butadiene in indoor environments are similar to the concentrations in the local ambient air.

Higher concentrations of butadiene have been measured in indoor air where ETS was known to be present. However, the data on concentrations of butadiene in ETS-contaminated indoor air are highly variable and are not sufficient to reasonably define the range of mean concentrations. Nevertheless, there is a high degree of certainty that non-smokers spending a considerable proportion of their time in indoor environments where ETS is present are exposed to higher concentrations of butadiene than are non-smokers who are not exposed to ETS. There is a high degree of certainty that smokers are exposed to higher concentrations of butadiene and have significantly higher daily intakes than do non-smokers. However, there are no reliable, recent data on the content of butadiene in the mainstream smoke of Canadian cigarettes.

There is somewhat less certainty that butadiene monomer is not released in detectable amounts from consumer products (e.g., synthetic materials) incorporating this compound in their production. Although there may be contributions to indoor concentrations of butadiene from certain cooking activities, the data are not sufficient to identify specific sources or activities or to identify a range of emissions of butadiene during cooking.

Although data on levels of butadiene in foodstuffs are scarce, based on the physical and chemical properties of the substance and the fact that it is released primarily to ambient air (where it is likely to remain without partitioning to other media), there is a reasonable degree of certainty that food does not represent a major source of exposure. Similarly, although the database for concentrations of butadiene in drinking water is limited, there is a reasonable degree of certainty that drinking water is not an important source of exposure for the general public in Canada, based on the volatility and release patterns of the compound.

There is some degree of uncertainty that the weight of epidemiological evidence for the association between butadiene and leukemia satisfies criteria for causality. In particular, the need for coherence is seemingly not addressed, since the observed increase in mortality due to leukemia in styrene-butadiene rubber workers was not observed in the cohorts of monomer workers (although there was some evidence of an association with other forms of lymphohematopoietic cancer, particularly in short-term workers). This may be related to the nature of exposure to both butadiene and other substances in these two industries. However, in view of the overwhelming evidence of carcinogenicity and genotoxicity in experimental animals, available information on species differences in sensitivity likely being related to differences in metabolism and the potential for considerable interindividual variability in metabolism to putatively toxic metabolites in the human population, along with the limited evidence of genotoxicity in occupationally exposed populations, there is a high degree of confidence that butadiene is likely to be carcinogenic in humans. Based on the extensive database on the genotoxicity of butadiene and its principal metabolites both *in vitro* and *in vivo* in both somatic and germ cells, confidence that butadiene

induces tumours (and possibly other effects) through direct interaction with genetic material is high.

Although the assessment of the exposure of the critical cohort of workers is likely one of the most comprehensive published to date, there is also uncertainty in the estimates of carcinogenic potency derived on the basis of this study, due primarily to the fact that the estimates of exposure are based on only a limited number of actual historical monitoring data.[9] For example, when the exposure of workers at one plant was re-examined, there were two- to threefold changes in the estimates for several job groups (with a 10-fold increase for one job group). In addition, with the exception of incorporating exposure to styrene as a stratification variable in the analyses, potential interactions between various occupational exposures could not be taken into account in the derivation of the carcinogenic potency based on the observations in this cohort. It has also been demonstrated that genetic polymorphism for several of the enzymes involved in metabolism of butadiene affects sensitivity to toxic effects induced by the substance. Also, since information on genotype for the relevant enzymes was not available for this large cohort and only a small amount of information on the distribution in the general population has been identified, it is not possible to determine how representative the study cohort is of the genetic susceptibility to butadiene of the general public.

With respect to the quantitation of exposure–response and derivation of potency estimates based on the epidemiological data, the inability of any of the models to consistently predict leukemia rates in the validation study contributes to additional uncertainty. In addition, the small number of leukemia cases being modelled contributes to model instability. However, the fact that the range of potency estimates for the four models is narrow (i.e., 1.4–4.3 mg/m^3) increases the confidence in the calculated potencies.

In view of the likely variability in metabolism of butadiene across the human population related to genetic polymorphism for relevant enzymes, estimates of carcinogenic potency as well as benchmark concentrations for non-cancer effects based on studies in mice are considered justifiably conservative. However, because of the high mortality in the study in mice in which exposure–response could best be characterized and the limitations in the study in rats (high mortality at the higher of only two widely spaced exposure levels), there is a moderate degree of uncertainty in estimates of carcinogenic potency derived on the basis of investigations in experimental animals. It is noteworthy that if the calculated margins between exposure and carcinogenic potency presented above that serve as a basis for prioritization of options to reduce exposure were derived on the basis of the 95% LCLs of the TC_{05}s for tumours in mice, the values would differ by only 1.4- to 3.3-fold (i.e., within the same order of magnitude) from those calculated on the basis of the point estimates; similarly, use of the 95% LCLs of the TC_{05}s for tumours in rats would result in a 1.1- to 6.4-fold difference in the margins between exposure and potency. Also, it should be noted that, although these margins and measures of risk (EPIs) presented above were based on comparison of the 95th percentile of the exposure data for each scenario, use of the median concentration (i.e., the 50th percentile) and either the point estimates of carcinogenicity or the associated 95% LCLs would result in a fivefold difference in the resulting values for the general population and a 10-fold difference in values for those in an area influenced by a point source. However, for almost all exposure scenarios, the priority for

[9] Although it has not been possible to quantitatively characterize uncertainty regarding these estimates of exposure and the impact of this uncertainty upon the estimates of carcinogenic potency, data being collected currently may permit a more quantitative characterization in future (Lynch, 1998).

investigation of options to reduce exposure would remain moderate to high.

There is uncertainty about the relevance of the ovarian atrophy observed in mice to humans, based on lack of data on the relative role of butadiene in the etiology of these lesions. As a result, quantitative measures of dose–response developed on this basis must necessarily be interpreted with caution. In addition, the BMC_{05} presented above was based on inclusion of ovarian atrophy of all severities, including "minimal" severity, the biological significance of which is unclear. If only lesions of moderate or marked severity are considered, the resulting BMC_{05} and hence the calculated margin between exposure and effect level and EPIs would differ by about fivefold. (N.B.: Use of the 95% LCLs of the BMC_{05}s for atrophy of all severities or of only moderate or marked severity would result in only a 1.5- or 3-fold difference in the measure of risk.) However, in view of the weight of evidence of causality for the association between butadiene and these effects in mice and the relatively low value for the measure of dose–response compared with that for other types of effects, additional investigation in this area is deemed to be of high priority.

3.4 Conclusions

CEPA 1999 64(a): Based on analyses of the worst-case situations that could likely be encountered in Canada, risk quotients for water, air and soil are less than 1. The environmental risks associated with concentrations of butadiene likely to be found in Canada therefore appear to be low. Based on available data, it has been concluded that it is unlikely that butadiene is entering or may enter the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity. Therefore, butadiene is not considered to be "toxic" as defined in Paragraph 64(a) of CEPA 1999.

CEPA 1999 64(b): Butadiene is not involved in depletion of stratospheric ozone and likely does not contribute significantly to climate change. Based on its abundance and reactivity in air, it plays a role, along with other reactive volatile organic chemicals, in tropospheric ozone formation. Therefore, based on available data, it has been concluded that butadiene is entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends. Therefore, butadiene is considered "toxic" as defined in Paragraph 64(b) of CEPA 1999.

CEPA 1999 64(c): Available data support a plausible mode of action for induction of tumours (and possibly related reproductive effects, although data are inconclusive in this regard) by butadiene involving direct interaction with genetic material. On this basis, butadiene is considered to be "toxic" as defined in Paragraph 64(c) of CEPA 1999. This approach is consistent with the objective that exposure to compounds where induction of cancer

(and possibly other effects) through direct interaction with genetic material is likely be reduced wherever possible and obviates the need to establish an arbitrary "*de minimis*" level of risk for the determination of "toxic" under CEPA 1999. Based on comparison of estimates of exposure with the potency for leukemia in humans and cancer and non-cancer effects in experimental animals, and taking into consideration the degree of confidence in the database upon which the quantitative measures of toxicity were based, the overall priority for investigation of options to reduce exposure to butadiene in the general environment in Canada, based solely on potential adverse health effects, is considered to be moderate to high.

Overall conclusion:

Based on critical assessment of relevant information, butadiene is considered to be "toxic" as defined in Section 64 of CEPA 1999.

3.5 Considerations for follow-up (further action)

Butadiene contributes to the photochemical formation of ground-level ozone. It is recommended that key sources of butadiene be addressed, therefore, as part of management plans for volatile organic chemicals that contribute to the formation of ground-level ozone.

Based on comparison of estimates of exposure for the general population with the tumorigenic potency, the priority to investigate options to reduce exposure of butadiene in ambient air in the vicinity of the identified point sources is considered to be high while that from more dispersive non-point sources (identified herein primarily as transportation) is considered to be moderate to high. Investigation of concentrations and potential sources of butadiene in indoor air may also be warranted.

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APPENDIX A SEARCH STRATEGIES EMPLOYED FOR IDENTIFICATION OF RELEVANT DATA

Environmental assessment

Data relevant to the assessment of the entry, environmental fate and exposure, and environmental effects of butadiene were identified in original literature, review documents, and commercial and government databases and indices, including on-line searches conducted between January and May 1996 of the following databases: Aqualine (Water Research Centre, Buckinghamshire; 1990–1996), ASFA (Aquatic Sciences and Fisheries Abstracts, Cambridge Scientific Abstracts; 1996), BIOSIS (Biosciences Information Services; 1990–1996), CAB (Commonwealth Agriculture Bureaux; 1990–1996), CESARS (Chemical Evaluation Search and Retrieval System, Ontario Ministry of the Environment and Michigan Department of Natural Resources; 1996), Chemical Abstracts (Chemical Abstracts Service, Columbus, Ohio; 1990–1996), CHRIS (Chemical Hazard Release Information System; 1964–1985), Current Contents (Institute for Scientific Information; 1990–1992, 1996), ELIAS (Environmental Library Integrated Automated System, Environment Canada library; January 1996), Enviroline (R.R. Bowker Publishing Co.; November 1995 – June 1996), Environmental Abstracts (1975 – February 1996), Environmental Bibliography (Environmental Studies Institute, International Academy at Santa Barbara; 1990–1996), GEOREF (Geo Reference Information System, American Geological Institute; 1990–1996), HSDB (Hazardous Substances Data Bank, U.S. National Library of Medicine; 1990–1996), Life Sciences (Cambridge Scientific Abstracts; 1990–1996), NTIS (National Technical Information Service, U.S. Department of Commerce; 1990–1996), Pollution Abstracts (Cambridge Scientific Abstracts, U.S. National Library of Medicine; 1990–1996), POLTOX (Cambridge Scientific Abstracts, U.S. National Library of Medicine; 1990–1995), RTECS (Registry of Toxic Effects of Chemical Substances, U.S. National Institute for Occupational Safety and Health; 1996), Toxline (U.S. National Library of Medicine; 1990–1996), TRI93 (Toxic Chemical Release Inventory, U.S. Environmental Protection Agency, Office of Toxic Substances; 1993), USEPA-ASTER (Assessment Tools for the Evaluation of Risk, U.S. Environmental Protection Agency; up to December 21, 1994), WASTEINFO (Waste Management Information Bureau of the American Energy Agency; 1973 – September 1995) and Water Resources Abstracts (U.S. Geological Survey, U.S. Department of the Interior; 1990–1996). Reveal Alert was used to maintain an ongoing record of the current scientific literature pertaining to the potential environmental effects of butadiene.

In addition, a survey of Canadian industry was carried out under authority of Section 16 of CEPA (Environment Canada, 1997b, 1997c). Targeted companies with commercial activities involving more than 1000 kg of butadiene were required to provide information on uses, releases, environmental concentrations, effects or other data that were available to them for butadiene. Additional relevant information was obtained from industry, including representatives of the Canadian Petroleum Products Institute (CPPI). The CPPI Toxic Substances Task Force provided copies of their *Technical Dossier — 1,3-Butadiene* (CPPI, 1997). Data obtained after March 1998 were not considered in this assessment unless they were critical data received during the 60-day public review of the report (October 2 to December 1, 1999).

Health assessment

A summary of data relevant to assessment of the potential risk to human health associated with exposure to butadiene was prepared in 1994 by BIBRA International. Additional and more recent data have been identified through searching the following databases using the chemical name or the CAS number: Cancerline (≥1992), Current Contents (Institute for Scientific Information; ≥1995), EMBASE (on-line version of Excerpta Medica, Elsevier Science; ≥1985), EUCLID (≥1994), Medline (U.S. National Library of Medicine; ≥1966), Toxline Plus (U.S. National Library of Medicine; ≥1993) and TOXNET (CCRIS, Chemical Carcinogenesis Research Information System, U.S. National Cancer Institute; GENE-TOX, Genetic Toxicology, Office of Toxic Substances, U.S. Environmental Protection Agency; and EMIC, Environmental Mutagen Information Center database, Oak Ridge National Laboratory; 1997). Numerous provincial and federal government officials and representatives of various industrial sectors were contacted between February and August of 1996 for data relevant to exposure and/or effects. In addition, a search of the databases Chemical Abstracts, EMBASE, EMIC, RTECS (Registry of Toxic Effects of Chemical Substances, U.S. National Institute for Occupational Safety and Health), Scisearch and Toxline Plus (≤1998) was conducted in order to identify information on the potential carcinogenicity of dimethyldithiocarbamate, in response to the suggestion by peer reviewers that this substance was a possible confounder in critical epidemiological investigations on the health effects of butadiene. Only data identified prior to April 1998 were considered in the determination of whether butadiene is "toxic" to human health.